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# Transgenic Fish Research: A Bibliography



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# Transgenic Fish Research:

## A Bibliography

A selected bibliography of research in the field of molecular biology and genetic engineering using fresh water fish.

by Robert D. Warmbrodt  
and Virginia Stone

Biotechnology Information Center  
Reference and User Services Branch  
Public Services Division  
National Agricultural Library  
United States Department of Agriculture  
Beltsville, Maryland 20705-2351

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## **Introduction**

The Biotechnology Information Center at the National Agricultural Library (NAL), in cooperation with NAL's Aquaculture Information Center, has compiled this bibliography of research and development in transgenic fish. This relatively new area of research represents a vigorous program to extend the use of molecular biology and genetic engineering techniques to help develop disease resistance, enhance reproduction, and increase the productivity of freshwater fish such as salmon, trout, catfish, and carp. Freshwater fish represent a multi-million-dollar industry in the United States, and thus their importance to the economy of local communities as well as to the national economy cannot be over-emphasized. In addition to the more practical aspects of transgenic fish development, the research generated using these organisms contributes significantly to an overall increase in the understanding of the molecular biology, gene sequencing, and gene expression and regulation of freshwater fish. In the broader context, the results will also undoubtedly enhance work using unrelated species such as domesticated plants and animals as well as micro-organisms.

This bibliography has been divided into several sections corresponding to the needs and interests of those involved in transgenic fish research and regulation. For an overview, the first section deals with general aspects of current transgenic fish research, followed by specific sections on gene sequencing and gene expression, immunology and diseases, and breeding and production. The final section deals with field release studies and the current status of the risk assessment and environmental impact of such studies.

The citations in this bibliography were extracted from three major databases including the National Agricultural Library's AGRICOLA database, the Aquatic Sciences and Fisheries Abstracts, and BIOSIS Previews. Each citation includes the title, author, and source as well as a listing of keywords. If available on the pertinent database, an abstract is also included with the citation. Finally, the National Agricultural Library Call Number is included if the material is part of the NAL collection.

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ROBERT D. WARMBRODT  
COORDINATOR, BIOTECHNOLOGY INFORMATION CENTER  
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## SAMPLE CITATIONS AND USER'S GUIDE

An explanation of citations of a sample journal article and monograph used in this bibliography appear below.

### Journal Article:

	<i>Title</i>	<i>Journal Title</i>	<i>Volume</i>	<i>Issue</i>	<i>Pages</i>	<i>Date</i>
<i>Authors</i>	<i>Transgenic Fish</i> Chen, T.T.; Powers, D.A.					
		<b>Source:</b> TRENDS IN BIOTECHNOLOGY 8(8):209-215 (1990).				

**Language:** English

*NAL Call No.* DNAL Call No.: TP248.13.T72

**Descriptors:** DNA; genes; mass culture; genetics; fish culture

#### **Abstract:**

A range of transgenic animal species have been generated using DNA microinjection, and application of this technique to fish is now showing some degree of success. Studies to optimize microinjection techniques specifically for use with fish, and to investigate possible alternative methods for mass culture, should lead to the commercial production of transgenic fish able to transmit desirable characteristics, such as enhanced growth or disease resistance, to their progeny.

### Monograph:

	<i>Publisher</i>	<i>Place of Publication</i>	<i>Date</i>	<i>Volume</i>	<i>Number of pages</i>
<i>Title</i>	<i>Genetics in Aquaculture III. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988</i>				
<i>Author</i>	Gjedrem, T., ed.				
	<b>Source:</b> Elsevier Science Publishers, Amsterdam, 1990, vol. 85, 333 pp.				

**Language:** English

*NAL Call No.* DNAL Call No.: SH151.I54 1988

**Descriptors:** genetics; fish culture; aquaculture techniques; biotechnology; conferences; selective breeding; immunology; underutilized species

#### **Abstract:**

A total of 27 papers and 29 posters are presented in sessions on biotechnology (gene technology, gene markers, immunology), breeding plans (phenotypic and genetic parameters, selection, and ploidy management and performance), and new fish species for aquaculture. Each contribution has been catalogued and indexed separately.



# Transgenic Fish Research

## General Research

1

*Transfection of the Dominant Selective Marker PSV2NEO into a Fish Cell Line Epithelioma Papulosum Cyprini EPC*

Araki, K.; Nagoya, H.; Onozato, H.

Source: BULLETIN OF THE NATIONAL RESEARCH INSTITUTE OF AQUACULTURE no. 20:1-9 (1991).

Descriptors: aquaculture; cell culture method; laboratory method; genetic engineering; biotechnology; calcium phosphate; electroporation; protoplast fusion

Language: English

Abstract:

The synthetic plasmid, pSV2neo, was transfected into a fish epithelium cell line (EPC) by calcium phosphate, electroporation, protoplast fusion and DEAE-dextran methods, which are used for mammalian cultured cells, to determine the optimum transfection method applicable for cultured fish cells. The efficiency of transfection was estimated by counting the number of colonies of stable transformants in a selection medium. 42, 26, and 21 transformants/5 multiplied by 106 cells per 30  $\mu$ g plasmid DNA were obtained by the calcium-phosphate, electroporation, and protoplast fusion methods, respectively. Southern hybridization indicated that the isolated stable transformants had a complete neo gene associated with the promoter and enhancer of SV40. These results suggest that pSV2neo was successfully integrated into the genome of the host fish cells by these methods and that the early promoter and enhancer region of SV40 can function in cultured fish epithelium cells.

2

*The New Endocrinology its Scope and its Impact*

Bern, H.A.

Source: AMERICAN ZOOLOGIST 30(4):877-886 (1990).

Language: English

DNAL Call No.: 410 Am3

Descriptors: review; transgenic fish; egg; embryonic development; autocrine; paracrine; hormone growth factor; pheromone; allelochemical; chemical mediation; molecular biology

3

*Transgenic Fish*

Chen, T.T.; Powers, D.A.

Source: TRENDS IN BIOTECHNOLOGY 8(8):209-215 (1990).

Language: English

DNAL Call No.: TP248.13.T72

Descriptors: DNA; genes; mass culture; genetics; fish culture

Abstract:

A range of transgenic animal species have been generated using DNA microinjection, and application of this technique to fish is now showing some degree of success. Studies to optimize microinjection techniques specifically for use with fish, and to investigate possible alternative methods for mass culture, should lead to the commercial production of transgenic fish able to transmit desirable characteristics, such as enhanced growth or disease resistance, to their progeny.

4

*Gene Transfer in Fish*

Chourrout, D.

**Source:** BULLETIN DE LA SOCIETE ZOOLOGIQUE DE FRANCE EVOL ZOOL 116(2):151-158 (1991).

**Language:** French

**Descriptors:** review; potential aquacultural genetic improvement; exogenous gene incorporation; cytoplasmic injection; transgenic fish production; method analysis

**Abstract:**

Biological experimentation in fish, as well as their genetic improvement for aquaculture, could benefit from technology of gene transfer in vivo, if convenient methods lead to the stable incorporation and expression of exogenous genes in these animals. Diverse constraints have determined the choice of cytoplasmic injection to introduce purified DNA into fertilized eggs, although other methods have also been tested with some success. Cytoplasmic injection has proved efficient in producing transgenic fish. Satisfactory expression levels of the transgenes remain a challenge, especially when heterologous genes are used.

5

*Techniques for the Development of Transgenic Fish a Review*

Chourrout, D.; Guyomard, R.; Houdebine, L.M.

**Source:** UCLA (UNIVERSITY OF CALIFORNIA LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY NEW SERIES, VOL. 116. TRANSGENIC MODELS IN MEDICINE AND AGRICULTURE; SYMPOSIUM, TAOS, NEW MEXICO, USA, JANUARY 28-FEBRUARY 3, 1989. R.B. Church, ed. Wiley-Liss, New York, 1990, pp. 89-100.

**Language:** English

**Descriptors:** oocyte; germinal vesicle; injection; genome integration; foreign gene expression; genetic engineering

6

*First International Symposium on Marine Molecular Biology Held at the Center of Marine Biotechnology University of Maryland, Baltimore, Maryland, USA on October 9-11 1988.*

Collodi, P.

**Source:** CYTOTECHNOLOGY 2(3):239-242 (1989).

**Language:** English

**DNAL Call No.:** QH585.C97

**Descriptors:** meeting; report; invertebrate; fish; bacteria; cell culture; gene expression; fish pathology; environmental signals; aquaculture

7

*Transgenesis in Fish Applications in Biotechnology*

De La Fuente, J.; Hernandez, O.; Guillen, I.; Castro, F.O.; Aguilar, A.; Herrera, L.; Uliver, C.; Perez, A.

**Source:** BIOTECNOL APL 8(2):123-139 (1991).

**Language:** Spanish

**Descriptors:** genetic manipulation; agricultural significance

8

*Chromosome-mediated Gene Transfer in Rainbow Trout*

Disney, J.E.

**Source:** DISSERTATION ABSTRACTS INTERNATIONAL PT. B - SCIENCE & ENGINEERING 49(11), 1989, 91 pp.

**Language:** English

**DNAL Call No.:** Z5055.U49D53

**Descriptors:** chromosomes; fish eggs; fish larvae; induced breeding; biotechnology; genetics; biological fertilization

**Abstract:**

Chromosome-mediated gene transfer is generally considered an *in vitro* technique whereby genes are transferred between cell lines on isolated metaphase chromosomes. The authors developed an *in vivo* chromosome-mediated gene transfer technique in order to assess whether stable, active genes could be transferred between species at the whole organism level. The technique involved fertilizing albino rainbow trout (*Salmo gairdneri*) eggs with gamma-irradiated brook trout (*Salvelinus fontinalis*) sperm and then heat-shocking the eggs to induce second polar body retention. Electrophoretic analyses of over 500 newly hatched transgenic offspring confirmed the expression of paternal Ldh-4, Mdh-3,4, Mdh-2, Aat-1,2, Gpi-3 and 6-Pgd; the transfer frequency of these loci varied from 2-17%. Cytogenetic analyses of embryos from these same crosses revealed variable numbers and types of chromosome fragments within individuals. Survival and pigment transfer in the resultant gynogenetic offspring varied considerably with the individual female used in a particular cross.

9

*Genetics in Aquaculture III. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Gjedrem, T., ed.

**Source:** Elsevier Science Publishers, Amsterdam, 1990, vol. 85, 333 pp.

**Language:** English

**DNAL Call No.:** SH151.I54 1988

**Descriptors:** genetics; fish culture; aquaculture techniques; biotechnology; conferences; selective breeding; immunology; underutilized species

**Abstract:**

A total of 27 papers and 29 posters are presented in sessions on biotechnology (gene technology, gene markers, immunology), breeding plans (phenotypic and genetic parameters, selection, and ploidy management and performance), and new fish species for aquaculture. Each contribution has been catalogued and indexed separately.

10

*Production of Stable Transgenic Fish by Cytoplasmic Injection of Purified Genes*

Guyomard, R.; Chourrout, D.; Houdebine, L.

**Source:** UCLA (UNIVERSITY OF CALIFORNIA LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY NEW SERIES, VOL. 87. GENE TRANSFER AND GENE THERAPY; TAMARRON, COLORADO, USA, FEBRUARY 6-12, 1988. Beaudet, A.L.; Mulligan, R.; and Verma, I.M., editors. Alan R. Liss, Inc., New York, New York, 1989, pp. 9-18.

**Language:** English

**DNAL Call No.:** QH506.V34 v.87

**Descriptors:** rainbow trout; plasmid; DNA; genetic engineering; promoters

11

*Gene Transfer in Fish. 34. Atlantic Fisheries Technological Conference and Seafood Biotechnology Workshop; St. John's, Newfoundland, Canada; 27 Aug-1 Sep 1989*

Hallerman, E.M.; Kapuscinski, A.R.; Hachett, P.B., Jr.; Faras, A.J.; Guise, K.S.



**Source:** ADVANCES IN FISHERIES TECHNOLOGY AND BIOTECHNOLOGY FOR INCREASED PROFITABILITY. Voigt, M.N. and Botta, J.R., editors, 1990, pp. 35-49.

**Language:** English

**DNAL Call No.:** SH334.55.A75 1989

**Descriptors:** fish culture; clones; genetics; breeding

**Abstract:**

Although research on gene transfer in fish was initiated just five years ago, at least 14 species of transgenic fishes have been produced by laboratories in at least 15 countries. Gene transfers in most experiments have been aimed at increasing growth rates through introduction of novel growth hormone genes. Some experiments have demonstrated stable germ-line transmission of novel genetic constructs, and accelerated growth has been observed among transgenic loach, carp, and northern pike. Technical advances in gene transfer in fish centre upon development of new expression vectors utilizing new regulatory elements or structural genes, which are frequently drawn from piscine genomes. Advances in gene transfer are illustrated by examples from our own experiments in goldfish (*Carassius auratus*) northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum vitreum*). Against the background of rapid technical advancement in gene transfer, interest in use of transgenic fish has grown rapidly within the aquaculture community.

12

*Transgenic Fish: Present Status and Future Directions. 1. International Symposium of Fish Endocrinology, Alberta, Canada, 12-17 June 1988*

Hew, C.L.

**Source:** FISH PHYSIOL. BIOCHEM. 7(1-6) PROCEEDINGS OF THE FIRST INTERNATIONAL SYMPOSIUM OF FISH ENDOCRINOLOGY. Peter, R.E.; Schreibman, M.P.; Pang, P.K.T.; Leatherland, J.F., editors, 1989, pp. 409-413.

**Language:** English

**Descriptors:** endocrinology; hormones; genetics; biotechnology

**Abstract:**

Successful production of transgenic fish by gene transfer technology is a very important breakthrough in the techniques of genetic manipulation in animals. This will have an impact of an unprecedented scale in fish biology, aquaculture and mariculture. This is a summary of the workshop on the Transgenic Fish presented at this Symposium. The Workshop discussed the current knowledge, experimental difficulties and related topics of the transgenic fish. It recommended further research on better gene constructs, methods development, safety containment and the closer collaboration of researches of different disciplines.

13

*Transgenesis in Fish*

Houdebine, L.M.; Chourrout, D.

**Source:** EXPERIENTIA 47(9): 891-897 (1991).

**Language:** English

**DNAL Call No.:** 475 Ex7

**Descriptors:** fish; embryos; hybridization; genes; DNA; experimental research; literature reviews

**Abstract:**

Gene transfer into fish embryo is being performed in several species (trout, salmon, carps, tilapia, medaka, goldfish, zebrafish, loach, catfish, etc.). In most cases, pronuclei are not visible and microinjection must be done into the cytoplasm of early embryos. Several reports indicate that the injected DNA was rapidly replicated in the early phase of embryo development, regardless of the origin and the sequence of the foreign DNA. The survival of the injected embryos was reasonably good and a large number reached maturity. The



proportion of transgenic animals ranged from 1 to 5 % or more, according to species and to experimentators. The reasons for this discrepancy have not been elucidated. In all species, the transgenic animals were mosaic. The data indicate that transgenesis in fish is relatively easy but that fish gene sequences must be preferably used to obtain a good expression of the transgenes. Fish is a good biological model, especially for developmental studies and it is an increasing part of human food. For these reasons, transgenesis in fish is most likely to be more and more practised in the coming years.

14

*Electroporation as a New Technique for Producing Transgenic Fish*

Inoue, K.; Yamashita, S.; Hata, J.I.; Kabeno, S.; Asada, S.; Nagahisa, E.; Fujita, T.

Source: CELL DIFFERENTIATION AND DEVELOPMENT 29(2):123-128 (1990).

Language: English

DNAL Call No.: QH607.A1C4

Descriptors: oryzias-latipes; mouse growth hormone; complementary DNA; metallothionein 1; promoter; gene transfer

**Abstract:**

A recombinant plasmid, pMV-GH, containing rainbow trout growth hormone cDNA fused to mouse metallothionein I promoter, was introduced into medaka (*Oryzias latipes*) by electroporation. Of 3109 fertilized eggs treated with electric pulses (750 V/cm, 50  $\mu$ s, 5 times), 783 (25%) hatched out. Four percent of the hatchlings were transgenic. To obtain transgenic lines, 180 hatchlings were maintained and 35 of them grew into adult fish. Two of these fish were transgenic. When one transgenic fish was mated with a normal female, the transgene was found in all the F1 offspring assayed. In F2 offspring obtained by mating transgenic F1 fish, 88% were transgenic.

15

*Production of Transgenic Medaka as a Model for Genetic Engineering of Fish*

Inoue, K.; Asada, S.; Kabeno, S.; Yamashita, S.; Nagahisa, E.; Fujita, T.

Source: PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC'89), 1989, p. 42.

Language: English

Descriptors: biotechnology; genetics; DNA; hormones

**Abstract:**

Techniques for foreign gene transfer into fish will be useful for breeding in aquaculture as well as for basic studies of genes. As a model for gene transfer studies in fish, a recombinant plasmid, pMV-GH containing rainbow trout (*Salmo gairdneri*) growth hormone cDNA fused to mouse metallothionein I promoter was introduced into medaka (*Oryzias latipes*). The plasmid sequence was transferred efficiently by microinjection into oocyte nuclei or into cytoplasm of fertilized eggs, and electroporation. The simplest method was electroporation though it was inferior to the two microinjection methods in gene transfer efficiency. Several transgenic individuals have already matured. DNA analysis of their offsprings to examine germ-line transmission of transgene is now in process.

16

*Integration of Transgenic Fish into Aquaculture*

Kapuscinski, A.R.

Source: FOOD REVIEWS INTERNATIONAL 6(3):373-388 (1990).

Language: English

DNAL Call No.: TX341.F662

Descriptors: review; genetic manipulation; novel genes; cost-effectiveness; environmental impact

17

*Transgenic Fishes (AFS Position Statement)*

Kapuscinski, A.R.; Hallerman, E.M.

Source: FISHERIES 15(4):2-5 (1990).

Language: English

DNAL Call No.: SH1.F54

**Descriptors:** genetics; DNA; chromosomes; ecological balance; man-induced effects; environmental impact; aquatic communities; biotechnology; control; fish; introduced species

**Abstract:**

The advent of gene transfer techniques has introduced the development of lines of fishes, as well as other aquatic organisms, bearing introduced genes. Because the performance and ecological impacts of transgenic organisms in natural ecosystems are unknown, uncontrolled release of transgenic fishes is undesirable. Public policies for regulating development and release of transgenic organisms are currently being formulated.

18

*Chromosome Manipulations in Tench (Tinca tinca L.) and Carp (Cyprinus carpio L.) in Czechoslovakia.; Conference on Fish Genetics and Breeding; Milenovice (Czechoslovakia); 18 Feb. 1988*

Linhart, O.; Slechtova, V.; Kvasnicka, P.; Rab, P.; Prikryl, I.

Source: PR. VURH VODNANY PAP. RIFH VODNANY, (18):53-60 (1989).

Language: English

**Descriptors:** selective breeding; biotechnology; genomes; monosex culture

**Abstract:**

Gynogenesis was induced in carp (*Cyprinus carpio*) and tench (*Tinca tinca*), respectively, using carp sperm with genome inactivated by exposure to Co super(60) gamma radiation at a dose of 1400 Gy. The spermatozoa remained normally motile after irradiation. Eggs were exposed to cold shock (0-4 degree C) in the period of the second meiosis: up to 41 % of the gynogenetic fry of carp and 21 % of the gynogenetic fry of tench were obtained after the shock. When the cold shock was used in the period of the second mitosis, the proportion of gynogenetic fry of carp increased to 0.94 %, compared with the control without cold shock (0.15 %). The gynogenetic progeny was female sex. The levels of recombination of 1.6-65 % in the 4 loci studied in gynogenetic carp and 29.7, 42.8 % in gynogenetic tench are in agreement with literature data on other fish.

19

*Preliminary Study on Total DNA Mediated Gene Transfer in Fish*

Liu, H.; Guo, W.; Wang, T.; Chen, H.

Source: ACTA HYDROBIOL. SIN. SHUISHENG SHENGWU XUEBAO 15(3):286-288 (1991).

Language: Chinese

**Descriptors:** DNA; biotechnology; sperm; fish; genetics

20

*The Application of Gene Manipulation to Aquaculture*

MacLean, N. and Penman, D.

Source: AQUACULTURE 85(1-4):1-20 (1990).

Language: English

DNAL Call No.: SH1.A6

**Descriptors:** transgenic fish production; genetics; DNA tagging

21

*Transgenic Animals*

Minhas, B.S. and Voelkel, S.A.

**Source:** BIOTECHNOLOGY: A COMPREHENSIVE TREATISE, VOL. 7B. GENE TECHNOLOGY. Jacobson, G.K. and Jolly, S.O., editors. VCH Publishers, Inc., New York, 1989, pp. 357-398.

**Language:** English

**DNAL Call No.:** QR53.B52

**Descriptors:** review; mammals; fish; poultry; recombinant DNA; gene transfer; technology; superovulation; in-vitro fertilization; embryo culture; embryo cloning; twinning; biotechnology

22

*Introducing Foreign Genes into Fish Eggs using Electroporated Sperm as a Carrier. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Mueller, F.; Ivics, Z.; Erdelyi, F.; Varadi, L.; Horvath, L.; MacLean, N.; Orban, L.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 91.

**Language:** English

**Descriptors:** DNA; genetics; fish eggs; sorption; biotechnology; sperm; fish culture

**Abstract:**

A new method has been developed for the introduction of foreign genes into fish eggs. The procedure is based on the incubation of fish sperm cells in DNA solution followed by the application of high field strength electric pulses (electroporation) to increase DNA binding and/or uptake. Gene expression assays and slot blot hybridization DNA analysis proved the presence of the transgene in 1 to 5% of several hundreds of examined larvae depending on the three fish species investigated. Southern blot and hybridization analysis are in progress to confirm these results. No transgene has been found so far in fries resulting from parallel experiments without electroporation. This is the first report on the successful application of electroporation of sperm cells for producing transgenic animals.

23

*Introduction of Carp Alpha-globin Gene in Rainbow Trout (Salmo gairdneri). 1. International Marine Biotechnology Conference (IMBC '89); Tokyo (Japan); 4-6 September 1989*

Oshiro, T.; Yoshizaki, G.; Takashima, F.

**Source:** PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC' 89). 1989, p. 41.

**Language:** English

**Descriptors:** genes; genomes; biotechnology; hemoglobins; fish eggs

**Abstract:**

Although transgenic fish have been experimentally produced in some laboratories, most of transferred genes were derived from mammals or procaryotes. In this study, the authors succeeded in the integration of carp alpha-globin gene (C alpha G) into the genome of rainbow trout by microinjection to the eggs. The DNA used for microinjection was the 2.2 kb BamHI-HindIII fragment. Cloning vector PBR 322 was eliminated before injection. Eggs were fertilized in 1mM glutathione solution (pH 8.0) which prevents the hardening of the chorion. About 10 super(7) copies of C alpha G were microinjected into the host cytoplasm between 3 and 7 hrs after fertilization. Preliminary results showed that C alpha G integrated into host genome of microinjected individuals.

24

*Transgenic Fish: Biological and Technical Problems*

Ozato, K.; Inoue, K.; Wakamatsu, Y.



**Source:** ZOOLOGICAL SCIENCE 6(3):445-457 (1989).

**Language:** English

**DNAL Call No.:** QL1.Z68

**Descriptors:** literature reviews; biotechnology; biological development; fish

**Abstract:**

Here the authors review the current state of transgenic research in fish. First, a brief description on the characteristics of fish in transgenic experiments and four lines of research in fisheries science and basic biology are provided. Then the usefulness of the medaka for studying gene expression in development is described referring to the authors' recent investigations.

## 25

*Failure of Sperm Cells as Vectors for Introducing Foreign DNA into Fish Eggs. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Paleudis, G.A.; Kohler, C.C.; Muhlach, W.L.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 78.

**Descriptors:** genes; sperm; fish eggs; DNA; genetics; methodology; growth

**Abstract:**

Hybrid tilapia *Oreochromis mossambicus* x *O. niloticus* spermatozoa were examined for their ability to take up recombinant bovine growth hormone DNA (pbGH/Sal I) and transfer the novel sequence to eggs. Three experiments were conducted, each of which had two treatment groups and a control group. One treatment in each experiment examined the ability of non-motile (dormant) tilapia spermatozoa to take up DNA. Spermatozoa were incubated for 4 h at room temperature in 2 µg/ml pbGH/Sal I DNA prior to fertilization of eggs. Incubation of spermatozoa with DNA did not adversely affect fertilization potential. Southern blot analysis of genomic DNA from fry produced by experimental sperm and DNA manipulations showed that none of the 42 individuals screened carried the foreign sequence. Under the constraints of the experimental conditions employed, tilapia spermatozoa apparently do not have the ability to take up heterologous DNA and transfer the novel sequence to eggs.

## 26

*Fish as Model Systems*

Powers, D.A.

**Source:** SCIENCE 246(4928):352-358 (1989).

**Language:** English

**DNAL Call No.:** 470 Sci2

**Descriptors:** evolution; selective breeding; biotechnology; fish; test organisms; experimental research

**Abstract:**

Fish represent the largest and most diverse group of vertebrates. Their evolutionary position relative to other vertebrates and their ability to adapt to a wide variety of environments make them ideal for studying both organismic and molecular evolution. A number of other characteristics make them excellent experimental models for studies in embryology, neurobiology, endocrinology, environmental biology, and other areas. In fact, they have played a critical role in the development of several of these disciplines. Research techniques that enable scientists to make isogenic lines in a single generation, create and maintain mutants, culture cells, and transfer cloned genes into embryos signal an increasing role for fish as experimental models.

27

*Biotechnology and Fish Aquaculture: Recent Aspects of this Research Developed in France. 1. International Marine Biotechnology Conference (IMBC '89); Tokyo (Japan); 4-6 September 1989*  
Prunet, P.; Chevassus, B.

**Source:** PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '89). 1989, p. 37.

**Language:** English

**Descriptors:** biotechnology; aquaculture; research programs; vaccines; hormones; fish culture; genetics; selective breeding

**Abstract:**

The development of biotechnology in general and recombinant genetic technology in particular have generated new trends of research in fish genetics, physiology and pathology. This presentation deals with recent French developments of this research. The following aspects are discussed: new methods in fish genetics such as chromosomes manipulation and gene transfer; obtention of new vaccines for fish from viral antigens expressed in bacteria; and use of recombinant hormones to modify some aspects of the physiology of the fish.

28

*Genome Manipulation in Fish: A Review. Conference on Fish Genetics and Breeding; Milenovice (Czechoslovakia); 18 February 1988*

Rab, P.; Linhart, O.

**Source:** PR. VURH VODNANY PAP. RIFH VODNANY (18):42-52 (1989).

**Language:** English

**Descriptors:** freshwater fish; genetics; selective breeding; genomes; biotechnology; fish culture

**Abstract:**

The review summarizes the basic principles and problems in the field of genome manipulations in fishes, which principally include induced gynogenesis and androgenesis, induction of triploidy, tetraploidy and higher ploidy levels, sex reversal and recently also transfer of genetic material (nuclei, active chromosome fragments and cloned genes). A list of recent literature added concerns fish species of the highest economic importance in Czechoslovakia, viz. *Cyprinus carpio* and *Salmo gairdneri*.

29

*Application of Biotechnology in Plant and Animal Systems*

Sarkar, A.N. and Natarajan, C.

**Source:** JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH 48(6):267-275 (1989).

**Language:** English

**DNAL Call No.:** 475 J82

**Descriptors:** tissue culture; crop improvement; biofertilizer; biogas; biologic pest control; forestry; fishery; animal production; multiple ovulation; embryo transfer; immunodiagnostics; vaccines; feed additives; waste treatment

30

*Using Microinjections of Exogenous DNA into Fish Eggs to Obtain Transgenic Animals*

Shakhbazyan, G.K. and Kazaryan, I.D.

**Source:** BIOLOGICHESKII ZHURNAL ARMENII 43(12):1020-1021 (1990).

**Language:** Russian

**DNAL Call No.:** 20 Er4

**Descriptors:** carp

31

*Fish Brain Peptides: Structures, Genes and Use in Aquaculture. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Sherwood, N.M.; Lovejoy, D.A.; Parker, D.B.; Coe, I.R.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 58.

**Language:** English

**Descriptors:** cyprinidae; salmonidae

**Abstract:**

The fish brain peptide, gonadotropin-releasing hormone (GnRH), is used for the induction of spawning and has the potential to stimulate body growth and gonadal development. Another neuropeptide, growth hormone-releasing hormone, is important in the control of growth and smoltification. The structure has been identified for carp and salmon. The brain hormone vasotocin, also identified in salmon, is thought to play a role in salt and water metabolism. The cDNAs and genes from each of these peptides hold considerable promise in the development of transgenic fish.

32

*Integration of Chromosome Set Manipulation and Transgenic Technologies for Fishes. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Thorgaard, G.H.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 63.

**Languages:** English

**Descriptors:** chromosomes; selective breeding; genetics; polyploids

**Abstract:**

A variety of chromosome set manipulation techniques can be applied which have implications for research with transgenic fish. Gynogenesis (induced all-maternal inheritance) can be used to generate isogenic lines and to map genes on chromosomes relative to their centromeres. Gynogenesis can also produce fish carrying extra chromosome fragments of foreign origin. Androgenesis (induced all-paternal inheritance) can be used to produce isogenic lines and to recover strains from cryopreserved sperm. Triploidy can be induced in fish by heat or pressure treatments of fertilized eggs and by crossing tetraploid individuals with normal diploids. Triploid fish are effectively sterile.

33

*Advances in Fisheries Technology and Biotechnology for Increased Profitability Papers from the 34th Atlantic Fisheries Technological Conference and Seafood Biotechnology Workshop, August 27 to September 1, 1989, St. John's, Newfoundland, Canada*

Voigt, M.N. and Botta, J.R., editors

**Source:** Technomic Publishing Company, Lancaster, 1990, 566 p.

**Language:** English

**DNAL Call No:** SH334.55.A75 1989

**Descriptors:** fishery technology - biotechnology

34

*Recombinant DNA Plasmids for the Study of Gene Transfer In Vivo and In Vitro in Rainbow Trout (*Oncorhynchus mykiss*). 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Welt, M.; Leung, F.C.



**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 91.

**Language:** English

**Descriptors:** fish culture; biotechnology; genetics; DNA; *Salmo gairdneri*

**Abstract:**

The study of gene transfer in fish *in vivo* and *in vitro* for growth enhancement and disease resistance requires recombinant DNA molecules containing several basic elements. To examine the correct transcription and translation of the exogenous genes we have made a number of recombinant constructs including two containing the bovine growth hormone (bGH) reporter gene, both genomic and cDNA ligated to a rainbow trout metallothionein B gene promoter (rtMT).

**35**

*The Tools of Genetics in Aquaculture*

Wohlfarth, G.

**Source:** ELLIS HORWOOD SERIES IN AQUACULTURE AND FISHERIES SUPPORT: MEDITERRANEAN AQUACULTURE; MEETING, BARCELONA, SPAIN, 1989. Flos, R.; Tort, L.; Torres, P., editors. Ellis Horwood Ltd., New York, 1990, pp. 167-180.

**Language:** English

**DNAL Call No.:** SH101.M46M4

**Descriptors:** fish; selective breeding; domestication; artificial selection; genetic engineering; aquaculture; fishing industry

## Gene Expression and Sequencing Studies

**36**

*Integration and Expression of Human Growth Hormone Gene in Teleostei*

Benyumov, A.O.; Enikolopov, G.N.; Barmintsev, V.A.; Zelenina, I.A.; Sleptsova, L.A.; Doronin, Yu K.; Golichenkov, V.A.; Grashchuk, M.A.; Georgiev, G.P.; et al

**Source:** GENETIKA 25(1):24-35 (1989).

**Language:** Russian

**DNAL Call No.:** QH431.A1G4

**Descriptors:** transgenic fish; plasmid DNA; RNA; embryogenesis; growth acceleration

**Abstract:**

Plasmid DNA containing human growth hormone gene was microinjected into cytoplasm of loach (*Misgurnus fossilis* L.) fertilized eggs. After plasmid injection, more than 50% of embryos reached the hatching stage. In control experiments embryogenesis was completed giving 72.5% of injected and 90% of intact larvae. Southern blot hybridization analysis revealed integration of injected recombinant DNA constructions into fish chromosome's DNA (2-30 copies per genome), without any significant rearrangements. Significant increase in length and weight of transgenic fish was observed in experiments ( $P < 0.01$ ). S1-analysis of RNA demonstrated correlation of the amount of specific RNA molecules and the accelerated growth of the individual specimens and proper utilization of the transcriptional start points in transgenic fish.

**37**

*Gene Transfer Expression and Inheritance of Rainbow Trout and Human Growth Hormone Genes in Carp and Loach*

Chen, T.T.; Lin, C.M.; Zhu, Z.; Gonzalez-Villasenor, L.I.; Dunham, R.A.; Powers, D.A.

**Source:** UCLA (UNIVERSITY OF CALIFORNIA LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY NEW SERIES, VOL. 116. TRANSGENIC MODELS IN MEDICINE AND AGRICULTURE; SYMPOSIUM, TAOS, NEW MEXICO, USA, JANUARY 28-FEBRUARY 3, 1989. Church, R.B., editor. Wiley-Liss: New York, 1990, pp. 127-140.

**Language:** English

**Descriptors:** fertilized egg; microinjection; large; transgenic fish; size; commercial; hormone; production; genetic engineering; biotechnology

**38**

*Expression of Homeotic Genes in Common Carp (Cyprinus carpio L.). 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Cheng, J.G.; Liao, C.F.; Hsu, Y.L.; Wu, J.L.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 92.

**Language:** English

**Descriptors:** DNA; fish culture; genetics; biotechnology

**Abstract:**

Genetic and molecular analyses of *Drosophila* development mutants led to the discovery of homeotic genes which control segmental identity in the fruit fly. All the known homeotic genes contain a conserved protein encoding DNA sequence of about 180 bp, named the homeobox, which is present in multiple copies in the genomes of most higher animal species. By using a *Drosophila* homeobox-containing gene (*Aptp*) fragment as a probe, Southern hybridization revealed that there are at least two homeobox-like genes in carp, tilapia and zebrafish. RNA slot hybridization showed that the *Aptp*-like gene(s) expressed intensively in the carp intestine, eye, kidney and heart, less intensively in the liver, testis and cerebellum, but not in the cerebrum, pituitary and muscle.

**39**

*Isolation and Characterization of Striped Bass GH cDNA Sequence. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Cheng, C.M.; Lin, C.M.; Chen, T.T.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 92.

**Language:** English

**Descriptors:** DNA; genetics; biotechnology; fish culture

**Abstract:**

We are studying the structure and regulation of growth hormone (GH) gene(s) in striped bass (*Morone saxatilis*). As a step toward this direction, a cDNA library was constructed from poly(A) super(+) RNA of striped bass pituitary glands. Five positive clones were isolated from this cDNA library using a 33mer synthetic oligonucleotides derived from the conserved region of GH polypeptides as a probe. The cDNA insert contains an open reading frame of 204 amino acid residues, 58 bp upstream of ATG codon, and 239 bp of 3' untranslated sequences. The predicted amino acid sequence shares 90% homology with the GH of bluefin tuna (*Thunnus thynnus*) and 60% with that of rainbow trout (*Oncorhynchus mykiss*).

**40**

*Expression and Fate of CAT Reporter Gene Microinjected into Fertilized Medaka *Oryzias latipes* Eggs in the Form of Plasmid DNA Recombinant Phage Particles and its DNA*

Chong, S.S.C. and Vielkind, J.R.

**Source:** THEORETICAL AND APPLIED GENETICS 78(3):369-380 (1989).

**Language:** English



**DNAL Call No.:** 442.8 Z8

**Descriptors:** puvscat; fish farming; chloramphenicol; acetyltransferase; genetic engineering

**Abstract:**

Fertilized medaka (*Oryzias latipes*) eggs were cytoplasmically injected with the chloramphenicol acetyltransferase (CAT) gene encompassed in supercoiled and linear plasmid DNA, as well as in intact recombinant phage particles and DNA isolated from the phage. Expression for the CAT plasmid DNA was highest at the gastrula/neurula stage, while for the DNA of the phage, it peaked in the 1-week old embryo; then expression declined but was still detectable in early adulthood (4 weeks post injection). Following the fate of exogenous DNA, an extensive replication was observed in early embryogenesis, and DNA was still found 4 weeks after injection, suggesting a possibility of integration. The system is useful as a transient expression system for the analysis of early developmental genes in particular, but also as a test system for the analysis of cloned genes of interest for the farming of economically important fish species. The fact that DNA transferred in intact phage particles or its DNA is functionally active opens the possibility to introduce larger DNA pieces (20 kb), e.g., for the functional test of larger and more distant control regions.

**41**

*Inheritance and Expression of Heterologous Genes Coupled with Ubiquitary and Tissue-specificity. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Chourrout, D.; Pilstrom, L.; Tewari, R.; Michard-Vanhee, C.; Perrot, E.; Thuvander, A.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 62.

**Language:** English

**Descriptors:** DNA; genes; fish eggs; genetics

**Abstract:**

The injection of linear DNA into the cytoplasm of trout eggs results in its transmission to a minority of F1 off-springs by mosaic adults. We produced seven F2 families in which the transgenes were found in mendelian proportions. Southern analyses clearly argued for their integration in five of them, but had to be complemented by several further investigations in order to rule out the possibility of an extrachromosomal persistence in the two others. Several constructs associating intronless coding sequences with heterologous enhancer/promoters were tested in this stable transgenic system.

**42**

*High-frequency Germ-line Transmission of Plasmid DNA Sequences Injected into Fertilized Zebrafish Eggs*

Culp, P.; Nusslein-Volhard, C.; Hopkins, N.

**Source:** PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA 88(15):7953-7957 (1991).

**Language:** English

**DNAL Call No.:** 500 N21P

**Descriptors:** genetics; DNA; fish eggs; methodology; mutations

**Abstract:**

With the goal of developing techniques for DNA insertional mutagenesis in zebrafish--we established procedures for rapidly obtaining and injecting large numbers of fertilized eggs. Using either of two plasmid constructs, we injected uncut DNA into fertilized eggs at the one- or two-cell stage. Fish hatched from injected eggs were raised to sexual maturity, and the frequency of transgenic founder fish was determined by pair-mating the fish and testing DNA extracted from pools of their 16-hr-old offspring by the polymerase chain reaction (PCR) and

then Southern analysis. Eggs injected with one of two different plasmids yielded no transgenic fish, but 7-25 % (19 of 115 overall) of the eggs injected with the other plasmid transmitted the injected sequences to their offspring (F<sub>1</sub>).

43

*Analysis of the Thyroid Hormone Receptor Genes in Zebrafish. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Essner, J.J. and Hackett, P.B.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 91.

**Language:** English

**Descriptors:** hormones; thyroid; fish culture; biotechnology; genetics; embryonic development

**Abstract**

We have shown in zebrafish (*Brachydanio rerio*) by Southern analysis that a number of different restriction fragments hybridize with probes produced from the rat c-erbA- alpha or c-erbA- beta cDNAs. Northern analysis of developing embryos reveals a 4.2 kb transcript hybridizing with both alpha and beta probes from 12 hrs to 4 days, a 2.1 kb transcript with the beta probe at 12 and 24 hrs of development, and a 1.6 kb message with the alpha probe only at 36 hrs post fertilization. Whole mount in situ hybridization with digoxigenin labeled probes produced from the rat c-erbA- alpha and beta cDNAs occurs in developing zebrafish as early as 6 hrs and up to 34 hrs post fertilization.

44

*An In Vivo Screen for Transgenic Fish Utilizing the Luciferase Reporter Gene. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Gibbs, P.D.L.; Peek, A.; Thorgaard, G.H.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 79.

**Language:** English

**Descriptors:** genes; luminescence; embryos; methodology

**Abstract:**

We have developed a convenient and inexpensive screen for transgenic fish expressing luciferase activity. The screen is a film assay for luminescence during embryonic development and has been accomplished using the zebrafish and rainbow trout. A transient assay of relative promoter strength of the SVO, SV2, RSV and CMV promoters in zebrafish shows the following hierarchy: CMV > RSV > SV2 > SVO. Optimization of screening conditions leading to maximum embryonic survival and a comparison of luciferase activity after circular or linear DNA injection will be discussed.

45

*Functional Analysis and Temporal Expression of Promoter Regions from Fish Antifreeze Protein Genes in Transgenic Japanese Medaka Embryos*

Gong, Z.; Hew, C.L.; Vielkind, J.R.

**Source:** MOL. MAR. BIOL. BIOTECHNOL. 1(1):64-72 (1991).

**Language:** English

**Descriptors:** embryos; genes; antifreeze proteins; biotechnology; cold resistance; temperature tolerance

**Abstract:**

Several series of sequences that are upstream of the transcriptional start site of different types of fish AFP genes were fused to the bacterial CAT gene, and their transcriptional role was examined in a transient expression assay after microinjection into Japanese medaka (*Oryzias latipes*) embryos at the 1-4 cell stage. Our studies demonstrated that the AFP genes have

functional promoter regions containing positive as well as negative regulatory regions, indicating that these genes could be regulated at multiple sites. We also observed a promoter-specific pattern of temporal expression. Typically, the CAT expression was low in the first 4 days of embryonic development or before the stage of body pigmentation, followed by a sharp increase. The high level was maintained until hatching (11-13 days after fertilization), by which time the activity decreased to a very low level.

46

*Integration and Germ Line Transmission of Foreign Genes Microinjected into Fertilized Trout Eggs*  
Guyomard, R.; Chourrout, D.; Leroux, C.; Houdebine, L.M.; Pourrain, F.

Source: BIOCHIMIE 71(7):857-863 (1989).

Languages: English

DNAL Call No: 383 SO1

Descriptors: genes; fish eggs; DNA; hormones; growth regulators; biotechnology

Abstract:

Persistence, integration into host genome, germ line transmission and expression of foreign genes microinjected into cytoplasm of fertilized rainbow trout eggs has been examined. Foreign DNA persisted as large random concatenates in approximately 50% of 6 to 12 month-old trout and exhibited a mosaic pattern between tissues. In some cases, free concatenates were observed indicating that extrachromosomal replication occurred in trout. 50% of the males had the foreign sequences in sperm DNA and all the examined animals transmitted these sequences to their progeny. The percentage of transgenic offsprings ranged from 10 to 30% and putative junction fragments were identified in Southern blot analysis in some of them. These results strongly support the hypothesis that the injected genes became integrated into the genome host, most likely after the first round of chromosomal replication.

47

*Analysis of Position Effects of Transcriptional Regulatory Elements in Transgene Expression in Cultured Cells and Transgenic Fish. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Hackett, P.B.; Liu, Z.; Moav, B.; Faras, A.; Kapuscinski, A.R.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 64.

Language: English

Descriptors: genes; genetics; DNA; molecular structure

Abstract:

Genes are transcriptionally regulated by specific binding of transacting protein factors to cis-acting DNA regulatory sequences. We have examined in detail the cis-acting enhancer and attenuator sequences in the beta-actin gene of carp. For this a large number of flanking and intronic elements were examined for their abilities to drive transcription of the chloramphenicol acetyl transferase (CAT) gene of bacteria. In particular, the serum responsive element (CC(A/T)6GG) also known as the CArG box or SRE is repeated at least twice in the vicinity of the beta-actin gene, once in the proximal promoter about 50 bp upstream of the transcriptional start site and once in the first intron. We have found, using linker replacements of two different lengths, that the SRE in the proximal promoter was not required for promoter activity in cultured fish cells, but was required in conjunction with the intronic SRE to give full expression in transgenic embryos.



48

*Characterization of AluI Repeats of Zebrafish (Brachydanio rerio)*

He, L.; Zhu, Z.; Faras, A.J.; Guise, K.S.; Hackett, P.B.; Kapuscinski, A.R.

**Source:** MOL. MAR. BIOL. BIOTECHNOL. 1(2):125-135 (1992).

**Language:** English

**Descriptor:** DNA; genetics; biotechnology

**Abstract:**

Two families of repetitive DNA sequences were isolated from the zebrafish genome and characterized. Eight different sequences were sequenced and classified by two standards, their (G + C) composition and their lengths. For convenience, the sequences were first divided into two types. Type I was (A + T)-rich, was repeated approximately 500,000 times, and constituted approximately 5% of the zebrafish genome. Type II was (G + C)-rich, was reiterated approximately 90,000 times, and comprised approximately 0.5% of the genome. Agarose gel electrophoresis of zebrafish DNA cleaved with AluI revealed three distinguishable bands of repetitive fragments; large (approximately 180 bp, designated RFAL), medium (approximately 140 bp, RFAM), and small (approximately 90 bp, RFAS).

49

*Analysis of the Adult Carp (Cyprinus carpio) Alpha-globin Genes by the Polymerase Chain Reaction.*

2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991

Hirono, I.; Miyata, M.; Hayashi, A.; Masuda, T.; Kobayashi, T.; Aoki, T.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 92

**Language:** English

**Descriptors:** genetics; enzymes; biochemical composition; chromosomes; blood; hemoglobins; fish culture

**Abstract:**

Carp (Cyprinus carpio) chromosomal DNA contains the 7 differences alpha-like globin genes. The No. 1 and No. 5 genes are probably pseudogene. The total RNA of adult carp were isolated from blood cells. Synthesis of the first strand of cDNA used an oligo(dT) or random primers and reverse transcriptase. Polymerase Chain Reaction condition is 30 cycles of denaturation at 94 degree C for 1 min, annealing at 55 degree C for 1 min, and extension at 72 degree C for 2 min. An approximately 400-bp fragment was detected. This fragment was characterized by restriction enzyme analysis. There are at least 2 differences genes were amplified.

50

*Regulation of Antifreeze Gene Expression in Winter Flounder and in Transgenic Fish Cells*

Huang, R.C.C.; Price, J.L.; Gurlie, B.

**Source:** UCLA (UNIVERSITY OF CALIFORNIA LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY NEW SERIES, VOL. 116. TRANSGENIC MODELS IN MEDICINE AND AGRICULTURE; SYMPOSIUM, TAOS, NEW MEXICO, USA, JANUARY 28-FEBRUARY 3, 1989. Church, R.B., ed. Wiley-Liss: New York, 1990, pp. 109-126.

**Language:** English

**Descriptors:** rainbow trout; bluegill; salmon season; temperature; photoperiod; messenger rna; genetic engineering

51

*Constitutive and Inducible Expression of a Transgene Directed by Heterologous Promoters in a Trout Liver Cell Line*

Inoue, K.; Akita, N.; Yamashita, S.; Shiba, T.; Fujita, T.

Source: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 173 (3):1311-1316 (1990).

Language: English

Descriptors: genetics; genes; genotypes

**Abstract:**

Activities of heterologous promoters and enhancers in cultured rainbow trout liver cells were examined employing the bacterial chloramphenicol acetyltransferase gene as the reporter. SV40 promoter-enhancer and Rous sarcoma virus long terminal repeat directed constitutive expression at high levels. Moloney murine leukemia virus long terminal repeat and SV40 promoter combined with Adenovirus type 2 enhancer were also constitutively expressed. *Drosophila* Hsp70 promoter was activated when the transformed cells were cultured at 25 degree C, a higher temperature than the temperature normally used, in faithful response to heat shock.

52

*Expression of Reporter Genes Introduced by Microinjection and Electroporation in Fish Embryos and Fry. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Inoue, K.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 63.

Language: English

Descriptors: genes; fish eggs; genetics

**Abstract:**

To achieve the expression of foreign genes in transgenic fish, well-characterized regulatory elements are indispensable. The authors constructed plasmids containing various promoters and enhancers derived from fish and other animals and reporter genes such as the bacterial beta-galactosidase (lacZ) gene and the chloramphenicol acetyltransferase (CAT) gene. We introduced them into eggs of medaka and rainbow trout by several methods including microinjection and electroporation, and the expression in embryos and fry were estimated using the histochemical staining for beta-galactosidase activity or the CAT assay. As the result, it was found that the metallothionein promoters derived from rainbow trout and mouse were inducible by heavy metals and that several promoters and enhancers including the sequences of mammalian viruses were constitutively active.

53

*Stage-dependent Expression of the Chicken Delta -crystallin Gene in Transgenic Fish Embryos*

Inoue, K.; Ozato, K.; Kondoh, H.; Iwamatsu, T.; Wakamatsu, Y.; Fujita, T.; Okada, T.S.

Source: CELL DIFFERENTIATION AND DEVELOPMENT 27(1):57-68 (1989).

Language: English

DNAL Call No.: QH607.A1C4

Descriptors: embryonic development; genetics

**Abstract:**

To study the regulation of gene expression of vertebrate crystallin genes, the chicken delta-crystallin gene was introduced into a small freshwater fish, medaka (*Oryzias latipes*), which lacks this gene, and its expression was examined immunohistologically at several developmental stages before hatching. The gene expression was detected in the central fiber

cells of the lens at an early stage, showing a stage-dependent expression. In non-lens tissues, the expression was barely detectable before tissue differentiation. It first became substantial mainly in mesodermal tissues and then later in a greater variety of tissues, including ectodermal and endodermal ones. Thus, the non-lens expression of delta-crystallin was also stage-dependent, with the stage being dependent on the tissue type. These results from lens and non-lens tissues are discussed in relation to tissue differentiation and two categories of delta-crystallin expression.

54

*Integration and Germ-line Transmission of Human Growth Hormone Gene in Medaka Oryzias latipes. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Lu, J.K.; Chrisman, C.L.; Chen, T.T.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 77.

Language: English

Descriptors: hormones; growth; fish eggs; fish culture; aquaculture techniques; genetics

Abstract:

The structural human growth hormone (hGH) gene or hGH fused to the promoter/regulatory regions from the mouse metallothionien (MT), viral thymidine kinase, rat cholecystokinin, or chicken beta-actin (CBA) genes were microinjected, via the micropyle, into the cytoplasm of newly fertilized medaka eggs. Over 39% of the embryos reached hatching stage after DNA injection. About 14% of embryos injected with CBA-hGH transgene hatched one or two days earlier than embryos injected with other constructs and controls. Embryos injected with CBA-hGH gene also had a higher survival rate than did the other gene construct groups. Between several and 40% of injected fish contain hGH DNA as determined by polymerase chain reaction (PCR) analysis.

55

*Transgene Transmission and Expression in Rainbow Trout and Tilapia. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

MacLean, N.; Iyengar, A.; Rahman, A.; Sulaiman, Z.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 65.

Language: English

Descriptors: fish eggs; genetics; biotechnology

Abstract:

Gene constructs using either mammalian or fish-derived promoter sequences have been introduced into the genomes of both rainbow trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis niloticus*) by microneedle injection into blastodisc cytoplasm of fertilised eggs. Transgenic induction is detected in approx. 15% of fish hatching from injected eggs in either species. Germ line transmission has been achieved with a proportion of the adult transgenics and patterns of transmission to the F1 generation will be presented.

56

*Expression and Transmission of the Melanogenic Phenotype in the Orange-Colored Mutant of Medaka Fish Introduced with Mouse Tyrosinase Gene*

Matsumoto, J.; Akiyama, T.; Hirose, E.; Nakamura, M.; Yamamoto, H.; Takeuchi, T.

Source: SIXTY-SECOND ANNUAL MEETING OF THE ZOOLOGICAL SOCIETY OF JAPAN, OKAYAMA, JAPAN, OCTOBER 13-15, 1991. ZOOL SCI (TOKYO) 8(6):1081 (1991).

Language: English

Descriptors: abstract; electron microscopy; light microscopy; transgenic skin



57

*Nucleotide Sequences of the Carp (Cyprinus carpio) Alpha-globin Gene Family. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Miyata, M.; Hirono, I.; Aoki, T.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 92.

Language: English

Descriptors: cloning; genetics; fish culture; hemoglobins; nucleotides; blood; chromosomes

Abstract

The carp alpha-like globin genes have been isolated from a gene library of carp chromosomal DNA fragments inserted into bacteriophage Charon4A. The restriction enzyme mapping and Southern blot hybridization analysis indicate that there are 7 differences alpha-like globin genes. (These were numbered from 1 to 7 of EcoRI large fragment to small fragment.) These alpha-globin gene fragments of recombinant Charon4A were subcloned into pUC118 and 119 in E. coli MV1184. The nucleotide sequences of these genes were determined. These genes except No. 1 contain the three exons and two introns.

58

*Selection of Promoters for Gene Transfer in Fish. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Moav, B.; Liu, Z.; Groll, Y.; Kapuscinski, A.R.; Hackett, P.B.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 63.

Language: English

Descriptors: cyprinidae; carassius auratus; brachydanio rerio; pisces

Abstract:

A variety of gene constructs containing carp beta-actin regulatory sequences were tested for their ability to drive transient expression of chloramphenicol acetyl transferase (CAT), bacterial reporter gene in three fish cell lines: carp epithelial cells (EPC), rainbow trout hepatoma cells (RTH 149) and rainbow trout fibroblasts (RTG2). The initial selection for high, medium and low levels of promoter-enhancer activities upon transfection into fish tissue culture cells, was followed by microinjection of the same and other beta-actin containing constructs into zygotes of carp, goldfish and zebrafish. The levels of expression of the CAT reporter gene throughout development of young fingerlings, is being used as a measure for the potency of the beta-actin enhancer-promoter combinations in fish species in vivo.

59

*Isolation of Sex-specific DNA Sequences in a Fish. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Nakayama, I.; Foresti, F.; Schartl, M.; Tewari, R.; Chourrout, D.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 92.

Language: English

Descriptors: sex determination; fish culture; genetics

Abstract:

A major obstacle for the production of monosex populations in fish is the usual absence of both sex-linked markers and differentiated sex chromosomes. This can be resolved by the isolation of sex-specific DNA probes. Sex differences in the repetitive genomic DNA bands were not observed in agarose gels stained with ethidium bromide, in the rainbow trout

(*Oncorhynchus mykiss*), the common carp (*Cyprinus carpio*, Nile tilapia), *Oreochromis niloticus*, and *Leporinus elongatus* (Anostomidae). Southern hybridization with heterologous DNA probes (Bkm, hHPRT, ZFY, SRY) did not reveal any sex-specific band either.

60

*Exogenous Gene Transfer and Expression in Medaka Embryos. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Ozato, K.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 64.

**Language:** English

**Descriptors:** fish eggs; genetics; DNA; freshwater fish

**Abstract:**

The medaka is a small egg-laying freshwater teleost which has been widely used as a laboratory animal. Several inbred strains have been established. Generation time is 2-3 months. The spawning is daily and year-round under artificial conditions. Transparency of eggs is distinct advantage for embryological manipulation. Two exogenous plasmids were injected into the oocyte nucleus, which were detected in 7-day-old embryos. p delta C-1B contained genomic chicken delta-crystallin sequences and its own promoter, and was expressed in the lens tissues in a stage-dependent fashion. pmiwZ contained promoters and enhancers of RSV-LTR and chicken beta-actin gene, and was expressed in surface cells of embryos at blastula and gastrula stages and in the yolk sac cells at later stages.

61

*Foreign Gene Expression in Fish Development. 1. International Marine Biotechnology Conference (IMBC '89); Tokyo (Japan); 4-6 Sept 1989*

Ozato, K.; Inoue, K.; Kondoh, H.; Iwamatsu, T.; Wakamatsu, Y.; Fujita, T.; Okada, T.S.

**Source:** PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '89). 1989, p. 69.

**Language:** English

**Descriptors:** genes; bacteria; embryonic development; biotechnology

**Abstract:**

The chicken delta-crystallin gene containing no exogenous promoter sequences and the bacterial beta-galactosidase gene containing RSV and mouse actin promoter sequences were introduced into the medaka (*Oryzias latipes*), and their expression was examined at several developmental stages. The chicken gene was not expressed before the stage of the embryonic body formation, and was expressed stage-dependently in the lens and non-lens tissues. The bacterial gene was expressed at the blastula stage.

62

*Integration, Expression and Inheritance of Rainbow Trout Growth Hormone Gene in Carp and Catfish. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Powers, D.A.; Chen, T.T.; Dunham, R.A.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 65.

**Language:** English

**Descriptors:** genes; growth; fish culture

**Abstract:**

Microinjection of DNA is currently used as a standard method to generate transgenic animal species including fishes. This method offers the possibility of improving the genetic background of aquaculture important fish species. As a step toward this direction, we have



transferred rtGH1 and rtGH2 cDNA (fused to the LTR of avian RSV) to common carp and channel catfish by microinjection. About 35 % of the injected embryos survived at hatching, and of which more than 10% of the survivors have stably integrated the rtGH cDNA. These transgenic individuals not only expressed the rtGH gene but also grew, on the average, 20% larger than their non-transgenic siblings.

63

*Molecular Analysis of Naturally Occurring Allelic Variants of Lactate Dehydrogenase from the Teleost Fundulus Heteroclitus. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Powers, D.A.; Lauerman, T.; Bernardi, G.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 67.

**Language:** English

**Descriptors:** amino acids; clones; nucleotides; mutations; genetics; electrophoresis; enzymes; natural selection; biotechnology; fish

**Abstract:**

The authors have cloned and sequenced a series of allelic variants of the "heart type" lactate dehydrogenase (LDH-B) from the teleost *Fundulus heteroclitus* and found extensive genetic variation. Using site-directed mutagenesis, the authors have begun to alter amino acid residues and generate biosynthetic LDH-B in order to study the functional importance of each amino acid substitution. The authors have uncovered "cryptic" amino acid substitutions that significantly affect the kinetics and stability of LDH-B. Further studies on the significance of naturally occurring amino acid interchanges should shed light on the significance, or lack thereof, of amino acid substitutions in natural populations and the importance of natural selection at the molecular level.

64

*Expression of Human Growth Hormone Gene in Fertilized Eggs from Atlantic Salmon and Rainbow Trout. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 Jun 1988*

Rokkones, E.; Alestroem, P.; Skjervold, H.; Gautvik, K.M.

**Source:** AQUACULTURE 85(1-4):329 (1990).

**Language:** English

**DNAL Call No.:** SH1.A6

**Descriptors:** biotechnology; fish culture; genetics; growth regulators; immunology

**Abstract:**

The authors describe a method for microinjection of DNA into fertilized eggs from Atlantic salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*). A gene construct with the mouse metallothionein promoter fused to the structural gene of human growth hormone was microinjected either as plasmid or as a linear DNA fragment. Transcriptional activity, translation and secretion of a product immunoreactive to human growth hormone antisera was demonstrated. The injected gene sequence was detected at the same position as the chromosomal DNA when injected as plasmid or as linear DNA. After digestion with BamHI restriction endonuclease, the human growth hormone gene was excised from the high molecular weight DNA fraction, suggesting that the injected gene either was integrated into the host genome or was stably retained as high molecular weight concatemers.

65

*Characterization of Three cDNA of Insulin-like Genes from the Liver of Rainbow Trout. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Shamblott, M.J.; Chen, T.T.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 92.

**Language:** English

**Descriptors:** cloning; liver; DNA; genetics; fish culture

**Abstract:**

In order to understand the role of growth hormone in the regulation of insulin-like growth factor (IGF) expression in vertebrates, an attempt was made to isolate the cDNA and its genomic sequence for fish IGF. Double stranded cDNA was generated from the poly(A) super(+) -RNA of juvenile rainbow trout liver. This cDNA was used as a template in a polymerase chain reaction (PCR) and the PCR primers (29 mers) used were derived from two highly conserved regions of the insulin gene family (B and A regions). Two DNA fragments corresponding in size to those predicted from previously reported mammalian IGF-I cDNA were amplified and their nucleotide sequence determined.

**66**

*Evidence for Expression of Antifreeze Protein Genes in Transgenic Atlantic Salmon (Salmon salar). Annual Meeting 1989, Aquaculture Association of Canada Symposium; St. John's, Newfoundland Canada; 10 July 1989*

Shears, M.A.; Fletcher, G.L.; King, M.J.; Hew, C.L.; Davies, P.L.

**Source:** BULLETIN (AQUACULTURE ASSOCIATION OF CANADA) (89-3):22-24 (1989).

**Language:** English

**DNAL Call No.:** SH37.B8

**Descriptors:** fish culture; cage culture; genomes; antifreezes; cryobiology; proteins; temperature tolerance; marine aquaculture; cold resistance

**Abstract:**

We are attempting to produce a breed of more freeze-resistant salmon by transferring antifreeze proteins (AFP) genes to the genome of the Atlantic salmon (*Salmo salar*). The gene coding for the major AFP found in winter flounder (*Pseudopleuronectes americanus*) was microinjected into fertilized Atlantic salmon eggs. Stable integration and a low level of expression of the AFP gene was detected in a small percentage of salmon hatching from these eggs. Experiments are in progress to determine the inheritability of the transferred AFP gene and to increase the level of AFP expression in the transgenic salmon to attain physiologically significant amounts.

**67**

*Transfer, Expression, and Stable Inheritance of Antifreeze Protein Genes in Atlantic Salmon (Salmo salar)*

Shears, M.A.; Fletcher, G.L.; Hew, C.L.; Gauthier, S.; Davies, P.L.

**Source:** MOL. MAR. BIOL. BIOTECHNOL. 1(1):58-63 (1991).

**Language:** English

**Descriptors:** genes; cold resistance; temperature tolerance; biotechnology; fish culture; aquaculture techniques

**Abstract:**

Fertilized Atlantic salmon eggs were injected through the micropyle with 10 super(6) copies of winter flounder antifreeze protein gene under the control of its natural promoter. When blood from 2-year-old P sub(1) fish was assayed for the antifreeze protein gene by the polymerase chain reaction, 10 out of 324 fish (similar to 3%) were positive for the presence of the transgene. This was at least double the rate predicted from measuring gene expression by detecting antifreeze protein precursor in sera using immunoblotting. Inheritance and expression

of the antifreeze protein gene in the F1 generation were established by polymerase chain reaction assays and immunoblotting. A cross between P sub(1) transgenic male 1469 and a wild-type female produced 24 transgenic progeny out of 137 tested (17%).

**68**

*Molecular Cloning and Sequence Analysis of the Chum Salmon Growth Hormone Genomic Gene. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991* Shen, X.Z.; Wang, Y.; Welt, M.; Liu, D.M.; Leung, F.C.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 93.

**Language:** English

**Descriptors:** fish culture; biotechnology; growth regulators; hormones

**Abstract:**

The genomic DNA of chum salmon (*Oncorhynchus keta*) from Amur, China was isolated and partially digested by *Sau* 3A. The fragments of 15-21 kb range were inserted into the *Bam* HI sites of the phage EMBL3 vector. The genomic library of chum salmon was constructed with a capacity of  $3 \times 10^5$  plaque-forming units. For the screening of growth hormone (GH) gene, two 19-mer probes were synthesized representative of the coding regions which are conserved between chum salmon GH cDNA and rainbow trout GH genomic DNA. A clone with an insert of 16.8 kb in length was obtained. Restriction mapping and Southern blot analysis showed that it contained a full-length GH gene with several kilobases flanking both ends.

**69**

*Highly Sensitive Detection System for Luciferase Gene in Transgenic Fish. 1. International Marine Biotechnology Conference (IMBC '89); Tokyo (Japan); 4-6 Sept 1989*

Tamiya, E.; Masaki, K.H.; Sugiyama, T.; Karube, I.

**Source:** PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '89). 1989, p. 42.

**Language:** English

**Descriptors:** genes; biotechnology; enzymes; genetics; bioluminescence

**Abstract:**

Foreign gene transfer into fish is a powerful tool in establishment of experimental fishes and resources of fisheries. Luciferase assay offers very sensitive, rapid and inexpensive determination system for transcription and translation activities. Firefly luciferase catalyzes light production with energy transfer from ATP. A very sensitive light detection system consisted of photomultiplier tube, power supply and amplifier are linked to microcomputer. Maximum light emission in luciferase cloned *E. coli* was obtained within 0.3 sec. The authors microinjected the luciferase gene into nuclei of medaka (*Oryzias latipes*) oocytes. 5 of 17 microinjected oocytes showed luciferase activity. Luciferase expression is different in transgenic medaka.

**70**

*Spatial Imaging of Luciferase Gene Expression in Transgenic Fish*

Tamiya, E.; Sugiyama, T.; Masaki, K.; Hirose, A.; Okoshi, T.; Karube, I.

**Source:** NUCLEIC ACIDS RESEARCH 18(4):1072 (1990).

**Language:** English

**DNAL Call No.:** QD341.A2N8

**Descriptors:** genes; enzymes; genotypes; genetics



**Abstract:**

Gene expression is regulated in a temporal and tissue-specific manner during embryogenesis. The luciferase gene is used as a visible indicator for gene expression. We microinjected pRSV DNA containing firefly luciferase gene into nuclei of Medaka (*Oryzias latipes*) oocytes.

71

*cDNA Encoding P450 Aromatase and P450c17 Lyase of Medaka and Rainbow Trout; Their Expression and Cloning in the Ovary. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Tanaka, M.; Sakai, N.; Nagahama, Y.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 93.

**Language:** English

**Descriptors:** vitellogenesis; fish culture; biotechnology; genetics

**Abstract:**

In salmonid fishes, estradiol-17 beta and 17 alpha, 20 beta dihydroxy-4-pregnen-3-one (17 alpha, 20 beta-DP) are the two biologically important mediators of oocyte growth and maturation, respectively. We have shown that a distinct shift in the steroidogenic pathway from estradiol-17 beta to 17 alpha, 20 beta -DP occurs in the ovarian follicle cells immediately prior to final oocyte maturation. It has also been found that prior to oocyte maturation P450c17 lyase activity decreases, while hydroxylase activity remains high, leading to an increase in 17 alpha-hydroxyprogesterone production, the precursor of 17 alpha, 20 beta-DP biosynthesis, by the follicle cells. In this report, we have used human cDNA probes to clone cDNAs encoding P450 aromatase and P450c17 from cDNA libraries of medaka (*Oryzias latipes*) and rainbow trout (*Oncorhynchus mykiss*).

72

*The Structure of Catfish Growth Hormone and Prolactin Genes and Their Evolutionary Implications. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Tang, Y.; Lin, C.M.; Chen, T.T.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 93.

**Language:** English

**Descriptors:** fish culture; growth regulators; genetics; biotechnology; polypeptides

**Abstract:**

The ultimate objective of this research is to study the structures of catfish growth hormone (GH) and prolactin (Prl) genes and their evolutionary implications. DNA fragments of 1.6 and 1 kilo base (Kb) pairs generated by polymerase chain reaction (PCR), encoding part of the catfish GH and Prl genes respectively, were used as probes to screen a genomic library constructed in the lambda phage vector. Both GH and Prl genes are approximately 3.3 Kb in length, comprising five exons and four introns. The initiation codons, termination codons and canonical polyadenylation sequences of both genes were identified.

73

*Expression of Rainbow Trout Growth Hormone cDNA in Escherichia-coli from Vector Utilizing TAC Promoter*

Tsai, H.J.; Tseng, C.F.

**Source:** JOURNAL OF THE FISHERIES SOCIETY OF TAIWAN 19(1):45-53 (1992).

**Language:** English

**DNAL Call No.:** SH135.T3

**Descriptors:** fish growth rate; expression; plasmid; ribosomal binding site; shine-dalgarno sequence; RRRNB; transcription terminator; recombinant growth hormone; biotechnology; complementary DNA  
**Abstract:**

It has been demonstrated that the growth rate of cultured fish and shellfish can be enhanced by the recombinant growth hormone (rGH). In order to make rGH become economically feasible, we reconstructed a plasmid which expressed rainbow trout GH cDNA more efficiently than that was previously reported in pAF51. This new expression plasmid, pPROKRT, with 5.4 kilobase pairs, contains a tac promoter and a rrnB transcription terminator. The distance between the ribosomal binding site and the initial codon is 16 base pairs. The order of amino acid (aa) prior to the first aa, isoleucine, of mature GH polypeptide is Met-Gly-Gly-Ser-Ala. When proteins were extracted from cells and analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, one significant protein band with molecular weight of 22 kilodaltons were produced by Escherichia coli JM109 harboring the pPROKRT. This product was positively immunoreactive to the rabbit antiserum against natural chum salmon GH when Western blotting was employed. The amount of rGH produced from this strain was more than 7% of total cytosolic proteins, which was 7 times higher than that was produced by pAF51.

74

*Transgenic Medaka System. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Vielkind, J.R.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 78.

**Language:** English

**Descriptors:** genes; embryos; genetics; methodology; fish eggs

**Abstract:**

Many fish species should be ideal as transgenic systems because they are egg layers and the embryos are of large size and transparent. However, large yolk masses make the nucleus of the zygote invisible and thus alternative methods to nuclear microinjection of the zygote must be used. To test the feasibility of cytoplasmic injection of foreign genes, embryos of the Japanese medaka (*Oryzias latipes*) were injected at the 1-2 cell stage with the CAT reporter gene driven by a SV40-RSV double promoter-enhancer region encompassed in supercoiled and linear plasmid form, in phage DNA and in intact recombinant phage particles. The gene was expressed up to early adulthood paralleled by the survival of the injected DNA which was converted into high molecular weight form and replicated during early embryogenesis.

75

*Gene Transfer and Expression of the CMV-beta Gal Fusion Gene in Embryos of the African Catfish (Clarias gariepinus) and the Zebrafish (Brachydanio rerio). 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Volckaert, F.; Hellemans, B.; Daemen, E.; Ollevier, F.; Belayew, A.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 91.

**Language:** English

**Descriptors:** fish culture; biotechnology; genetics; DNA; enzymes

**Abstract:**

Our research focuses on the in vivo testing of homologous inducible and tissue-specific fish promoters. In a first step, we wish to set up optimal conditions for foreign gene injection in early embryos of the African catfish (*Clarias gariepinus*). Zebrafish (*Brachydanio rerio*) were used in parallel as control. Embryos of both fish were injected with 0.5 to 50 pg of linearised

CMV-beta Gal during the one and two-cell stage. A histochemical assay of beta Gal expression showed that in one experiment 40% of the catfish had a mosaic pattern at 24 h (no expression occurred before that time). Zebrafish showed 30% mosaic expression at 48 h (none earlier) and 100% total expression at 72 h.

76

*Gene Transfer Expression and Inheritance of PRSV-Rainbow Trout-GH Complementary DNA in the Common Carp Cyprinus-carpio linnaeus*

Zhang, P.; Hayat, M.; Joyce, C.; Gonzalez-Villasenor, L.I.; Lin, C.M.; Dunham, R.A.; Chen, T.T.; Powers, D.A.

Source: MOL. REPROD. DEV. 25(1):3-13 (1990).

Language: English

Descriptors: rous sarcoma virus-long terminal repeat; growth hormone; gene expression; transgenic fish; breeding technique

Abstract:

A recombinant plasmid containing the Rous sarcoma virus-long terminal repeat (RSV-LTR) promoter linked to rainbow trout (*Salmo gairdneri*) growth hormone (GH) cDNA was microinjected into fertilized carp eggs. Genomic DNA extracted from pectoral fin of individual presumptive transgenic fish was analyzed by dot blot and Southern blot hybridization, using the RSV-LTR and/or the GH cDNA sequences as probes. Out of 365 presumptive transgenic fish analyzed, 20 individuals were found to contain pRSV-rtGH-cDNA sequence in the genomic DNA. Expression of the trout GH polypeptide was detected by immunobinding assay in the red blood cells of nine transgenic fish tested. The level of expression, however, varied among the transgenics and could not be correlated with exogenous DNA copy number. Although there was considerable variation in the sizes of the transgenic fish, those microinjected during the one-cell stage were ( $P < 0.05$ ) 22% larger, on the average, than their sibling controls. A randomly selected fraction of the progeny derived from crosses between transgenic males and nontransgenic females inherited the foreign DNA. These transgenic progeny grew faster ( $P < 0.05$ ) than their non-transgenic siblings.

## Immunology and Diseases

77

*Reduction of IHNV Induced CPE by Transgene Expression of the Nucleocapsid Gene. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Anderson, E.D.; Leong, J.C.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 90.

Language: English

Descriptors: disease resistance; viral diseases; fish diseases; fish culture

Abstract:

Infectious hematopoietic necrosis virus (IHNV), a rhabdovirus, is a lethal virus of salmon and trout. The possibility of producing intracellular immunity to IHNV both *in vitro* and *in vivo* using transgene expression is being investigated. Fish tissue culture cells, RTG-2 and EPCs, were transfected with a full length cDNA copy of the nucleocapsid gene under the regulation of the MMTV LTR (an inducible promoter). Early experiments show that when the viral N gene is transcribed in the minus sense upon induction with dexamethasone in transfected cells, these cells are protected from IHNV induced CPE. The mechanism of protection is being investigated in the studies.



78

*The Application of Biotechnology in Fish Pathology in Japan. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Aoki, T.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 55.

**Language:** English

**Descriptors:** pathogens; DNA; genes; cloning; nucleotides; biotechnology; pathology; fish

**Abstract:**

The authors developed rapid identification methods by using DNA probe hybridization. A specific DNA fragment was cloned randomly from chromosomal DNA of *Edwardsiella tarda*, *Pasteurella piscicida*, *Streptococcus* sp., and *Vibrio anguillarum*. These probes only hybridized to themselves and did not hybridize to any other fish pathogens. The method is most useful for identification of each pathogen. Three hemolysin genes were cloned from *Aeromonas hydrophila* and three from *A. salmonicida*. No nucleotide sequence of an open reading frame (ORF) of the hemolysin genes was similar to itself or to the ORF of previously reported hemolysin genes from *Aeromonas*.

79

*Genetic Resistance to Microbial Disease in Fish*

Dutta, O.K.

**Source:** FISH. CHIMES 10(10):34, 51-52 (1990).

**Language:** English

**Descriptors:** fish culture; disease resistance; genetics; biotechnology; selective breeding; growth

**Abstract:**

A discussion is presented on fish culture and the development of fish strains with a hereditary capability for better growth and resistance to disease. Breeding trials and mating systems are examined briefly and details given of how to quantify a desired trait.

80

*Lysozyme from Rainbow Trout *Salmo gairdneri richardson* as an Antibacterial Agent Against Fish Pathogens*

Grinde, B.

**Source:** JOURNAL OF FISH DISEASES 12(2):95-104 (1989).

**Language:** English

**DNAL Call No.:** SH171.A1J68

**Descriptors:** hen; egg white; genetic engineering

**Abstract:**

The antibacterial effect of two lysozyme variants purified from rainbow trout, *Salmo gairdneri richardson*, kidney was investigated as part of a project directed towards increasing the disease resistance of fish by the transgenic technique. Seven bacterial strains from five Gram-negative species, of which one was considered non-pathogenic, were examined. One of the rainbow trout lysozymes was surprisingly potent, having substantial antibacterial activity on all strains tested. Hen egg-white lysozyme was bactericidal only against the one species considered non-pathogenic. The data suggest that lysozyme does play a role in the disease defence of rainbow trout and that the gene for the most active lysozyme may be suitable for testing the transgenic strategy.

81

*Specific DNA Probes for the Diagnosis of Bacterial Kidney Disease. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Leon, G.; Martinez, M.A.; Etchegaray, J.P.; Maulen, N.; Aruti, D.; Poblete, A.; Vera, I.; Figueroa, J.; Villanueva, J.; Krauskoff, M.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 90.

**Language:** English

**Descriptors:** DNA; fish culture; bacterial diseases; disease detection; fish diseases; fish eggs; biotechnology

**Abstract:**

Bacterial kidney disease (BKD), caused by the Gram positive diplobacillus *Renibacterium salmoninarum*, produces high mortality rates in the salmonid farming industry. Our main research goal is to devise molecular hybridization specific tests to diagnose BKD in asymptomatic fish and to identify stocks of eggs carrying the causative agent of BKD. In order to obtain specific DNA fragments which could be used as probes for the identification of the microorganism, we cloned selective DNA sequences of *R. salmoninarum*, by the PERT method. From 77 transformants, three recombinants were isolated which contain specific *R. salmoninarum* DNA sequences. The probes have been sequenced and used to diagnose BKD by in situ hybridization techniques and in the design of a PCR test for this disease.

82

*Biotechnologic Advances in Fish Disease Research. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Leong, J.C.; Anderson, E.; Bootland, L.; Drolet, B.; Chen, L.; Mason, C.; Mourich, D.; Trobridge, G.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 55.

**Language:** English

**Descriptors:** vaccines; biotechnology; disease control; fish culture; disease detection

**Abstract:**

The need for effective and commercially viable methods of diagnosing and controlling disease is clear. To meet this need, many different biotechnological approaches are currently being applied in the control of diseases in fish. These techniques have been applied in the development of control methods for infectious hematopoietic necrosis virus (IHNV) in salmon and trout. A study of the pathogenesis of IHNV in rainbow trout and the development of a subunit vaccine for IHNV will be presented to illustrate the use of these techniques in fish disease research.

83

*Influence of Seven Immunostimulants on the Immune Response of Coho Salmon to *Aeromonas salmonicida**

Nikl, L.; Albright, L.J.; Evelyn, T.P.T.

**Source:** DISEASES OF AQUATIC ORGANISMS 12(1):7-12 (1991).

**Language:** English

**Descriptors:** fish diseases; bacterial diseases; immunization; vaccines; drugs; therapy; biotechnology; fish culture

**Abstract:**

A study was conducted to evaluate the efficacy of 7 substances at potentiating a formalin-killed *Aeromonas salmonicida* bacterin in juvenile coho salmon *Oncorhynchus kisutch*. The substances were injected into the fish along with the bacterin. The fish were challenged 27 d



later with viable *A. salmonicida* cells by 2 methods (cohabitation and immersion). The cumulative mortalities in each of the experimental groups was then determined. A significant and consistent increase in protection over the groups receiving only the *A. salmonicida* bacterin was observed with 3 of the substances tested. These were VitaStim-Taito, lentinan and formalin-killed *Renibacterium salmoninarum* cells. One of these materials, VitaStim-Taito (a beta -1,3 glucan), showed particular promise for further studies.

84

*Bacterially Expressed Nucleoprotein of Infectious Hematopoietic Necrosis Virus Augments Protective Immunity Induced by the Glycoprotein Vaccine in Fish*

Oberg, L.A.; Wirkkula, J.; Mourich, D.; Leong, J.C.

Source: JOURNAL OF VIROLOGY 65(8):4486-4489 (1991).

Language: English

DNAL Call No.: QR360.J6

Descriptors: salmonid; trpE; fusion protein; enhanced resistance; genetic engineering; biotechnology

Abstract:

The ribonucleoprotein gene of infectious hematopoietic necrosis virus (IHNV) has been expressed in *Escherichia coli* as a trpE fusion protein. This viral protein does not induce protective immunity to lethal IHNV infection in fish, and virus-neutralizing antibodies do not react with this viral protein. However, when it was administered with a bacterial lysate containing a region of the IHNV glycoprotein, there was enhanced resistance in immunized fish to lethal virus infection.

85

*Contributo della Biotecnologia allo Sviluppo di Vaccini in Acquacoltura. Contribution of Biotechnology to the Development of Vaccines in Aquaculture*

Salati, F.; Ferrari, A.

Source: RIV. ITAL. AQUACOLT. 24(3):225-230 (1989).

Languages: English, Italian

Descriptors: fish culture; aquaculture techniques; vaccination; biotechnology; aquaculture development

Abstract:

This paper reviews the vaccines and biotechnology applied to aquaculture. The production and health of fish species will be improved in the next few years by the use of polyploid and/or transgenic fish, monoclonal antibodies, recombinant DNA, etc. However, biotechnological products must be safe for both animals and man.

86

*Virulence and Vaccine Efficacy of Vibrio anguillarum 775 Containing Site-Specific Mutations in the Virulence Plasmid pJM1. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Schmidt, K.A.; Hopper, C.A.; Singer, J.T.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 89.

Language: English

Descriptors: vaccines; vaccination; vibriosis; bacterial diseases; fish diseases; disease control; *Oncorhynchus mykiss*

Abstract:

The 65-kilobase(kb) virulence plasmid pJM1 codes for an iron sequestering system that is responsible for the high virulence phenotype of *Vibrio anguillarum* 775 in salmonid fishes. Specific plasmid-borne genes of interest encode pOM2, an outer membrane protein believed to be the cell surface receptor for ferric iron siderophore complexes, p40, a membrane-associated

polypeptide also believed to be required for iron uptake, and angR, a positive regulatory gene required for both pOM2 and siderophore biosynthesis. Results from initial protection studies indicate that live attenuated *V. anguillarum* 775 (pJM1-kan2) offers levels of protection greater than or equal to those obtained with commercially available killed bacterins while using 1,000-fold fewer cells.

**87**

*Stress Affects the Immune Response and Health of Fish in Aquacultural Systems. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Schreck, C.B.; Maule, A.G.; Slater, C.H.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 58.

**Language:** English

**Descriptors:** aquaculture; biological stress; immunity; stocking density; disease resistance; sex hormones

**Abstract:**

Aquacultural practices such as handling, crowding, and transportation evoke clinical signs of stress that are mediated through the endocrine system. We have found that brief exposure of salmonid fishes to stressors results in a transitory physiological stress response of rather short duration. However, the cortisol-induced suppression of the immune system may have detrimental consequences in terms of disease resistance perhaps weeks after the insult. Steroid hormones other than cortisol may also help regulate the immune system. Testosterone, a hormone that becomes elevated during maturation of both male and female salmonids, appears to be a major immunosuppressive agent.

**88**

*Vaccination Against Fish Rhabdovirus: Recent Advances. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Thiry, M.; Dheur, I.; Xhonneux, F.; Margineanu, I.; Dommes, J.; Vanderheijden,; Rossius, M.; Kinkelin, P. de; Renard, A.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 56.

**Language:** English

**Descriptors:** vaccines; fish culture; disease control; biotechnology; viral diseases; antibodies

**Abstract:**

The viral haemorrhagic septicaemia, VHS, is a devastating disease for the European fish farming industry. There is an urgent need for a vaccine to protect against this disease. The glycoprotein embedded in the envelope of the virus is the target of the neutralizing antibodies. This protein was produced by the recombinant DNA technology in bacteria, yeast and recently insect cells, using the baculovirus expression system. The recombinant proteins were administered by i.p. injection to 1 g rainbow trout. The protection provided by was measured after the challenge with the virulent VHS virus.

## Breeding and Production

**89**

*Fish GnRH Gene and Molecular Approaches for Control of Sexual Maturation. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Alestrom, P.; Klungland, H.; Kisen, G.; Andersen, O.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 65.

**Language:** English

**Descriptors:** genes; sex hormones; biotechnology; nucleotides; sexual maturity; fish

**Abstract:**

To examine the possibility of controlling the GnRH regulation of sexual maturation by molecular approaches, the following strategies were followed: GnRH genomic and cDNA sequences were isolated and characterized. By aligning the sequences from Atlantic salmon (*Salmo salar*) GnRH gene and cDNA, a similar general gene structure as seen in mammals was obtained. In the coding region for the prepro hormone, the GnRH domain was shown to be highly conserved while the signal peptide and GnRH-associated peptide (GAP) sequences were significantly diverged. Firefly Luciferase gene was utilized as selection marker for live transgenic model fish (zebrafish; *Brachydanio rerio* and medaka; *Oryzias latipes*). Genes for expression of antagonistic activity against GnRH were constructed for gene transfer to model fish.

90

*Induced Vitellogenesis in Triploid Coho Salmon (Oncorhynchus kisutch ). 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Benfey, T.J.; Dye, H.M.; Donaldson, E.M.

**Source:** AQUACULTURE 85(1-4):318 (1990).

**Language:** English

**DNAL Call No.:** SH1.A6

**Descriptors:** biotechnology; fish culture; genetics; polyploids; sex hormones; vitellogenesis

**Abstract:**

Small numbers of post-meiotic cells are produced by triploid salmonids. These develop to the mature stage in males (i.e., functional spermatozoa), but not in females. The aim of the present study was to examine whether impaired estrogen biosynthesis might be the cause of the lack of full maturation of post-meiotic oocytes in triploid females. Vitellogenesis was induced in immature diploid and triploid coho salmon (*Oncorhynchus kisutch*). Plasma vitellogenin levels were significantly higher in 17 beta estradiol (E sub(2))-injected fish than in sham-injected fish. There was no significant difference between triploids and diploids for the levels attained. E sub(2)-injected fish had significantly higher hepatosomatic indices and pituitary gonadotropin content than sham-injected fish by 4 weeks after the first treatment. These data suggest that the post-meiotic growth of oocytes in triploids is impaired due to an absent or diminished estrogen stimulus from the ovary on hepatic vitellogenesis.

91

*Biotechnology and Aquaculture the Role of Cell Cultures*

Bols, N.C.

**Source:** BIOTECHNOLOGY ADVANCES 9(1):31-50 (1991).

**Language:** English

**DNAL Call No.:** TP248.2.B562

**Descriptors:** review; fish; shellfish; seaweed; plant biotechnology industry; farming industry; genetic engineering

92

*Triploid Brown Trout (Salmo trutta) Produced by Hydrostatic Pressure Shock. 8. Annual Meeting of the Aquaculture Association of Canada; St. Andrews, New Brunswick, Canada; June 1991*

Brydges, K.; Benfey, T.J.

**Source:** BULLETIN (AQUACULTURE ASSOCIATION OF CANADA) 91(3):31-33 (1991).



**Language:** English

**DNAL Call No.:** SH37.B8

**Descriptors:** fish culture; genetics; biotechnology; hydrostatic pressure; polyploids; sterility

**Abstract:**

The goal of this study was to determine the optimum pressure treatments for the induction of triploidy in brown trout (*Salmo trutta*). Three variables were examined: duration of the pressure shock, magnitude of the pressure shock, and time after fertilization for start of the pressure shock. High yields of triploids were obtained from all treatments except those which were started within 15 minutes of fertilization. The optimum treatment found was in the range of 5.5 to 6.5 min at 9500 to 10500 psi, applied 25 to 30 min after fertilization at 8.5 degree C.

**93**

*Transgenic Zebrafish made by Electroporation; International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Buono, R.J. and Linser, P.J.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91), 1991, p. 91.

**Language:** English

**Abstract:**

We report here the generation of transgenic founder animals using an exponential decay electroporation system. The plasmid, RSVCAT, was linearized and introduced into fertilized zebrafish embryos at the two and four cell stage. This plasmid utilizes Rous Sarcoma Virus promoter to efficiently drive expression of chloramphenicol acetyl transferase in numerous types of eukaryotic cells. Sixty eight percent of the electroporated embryos survived to two days post hatching and beyond. Of these embryos 65% carried the transgene to some level and 30% were judged as strong positives based on signals generated via dot blot analysis.

**94**

*Studies on the Formation of Single Caudal Fin of Carassius auratus. 1. The Effect of Carp Egg-mRNA on the Development of the Goldfish Embryo*

Cai, N.; Yu, F.; Wu, X.; Xu, Q.; Xu, Y.

**Source:** OCEANOL. LIMNOL. SIN. HAIYANG YU HUZHAO 20(6): 508-513 (1989).

**Language:** Chinese, English

**Descriptors:** fins; organogenesis; embryonic development; RNA; genetics; phenotypes; experimental research

**Abstract:**

Poly (A) mRNA obtained from carp eggs was injected into goldfish *Carassius auratus* embryo at different developmental stages to study its effect on caudal fin development. The experiment consisted of two parts in which: (1) Microinjection of mRNA was made only once. The fertilized but uncleaved eggs were injected with carp egg-mRNA in the blastoderms, while all the other embryos were injected in the yolk sacs. The caudal fin transformation from double to single occurred in the embryos injected with the mRNA at 1-cell stage and in those injected with the mRNA at gastrula stage, or even at the auditory vesicle forming stage. The embryos with the mRNA introduced into the yolk sacs gave rise to more single caudal fin goldfish than those with the mRNA introduced into the blastoderm; and (2) Microinjection of mRNA was made several times. Some embryos were successively injected 3-5 times. Embryos injected with the mRNA successively formed more larva fish with single caudal fin than those injected with the mRNA only once. The foreign mRNA did not influence the host nucleus but, by translating into new proteins in the cytoplasm, it interfered in the factor regulating the double formation, resulting in single caudal fin formation.



95

*Cryopreservation of Sperm as a Means to Store Salmonid Germ Plasm and to Transfer Genes from Wild Fish to Hatchery Populations*

Cloud, J.G.; Miller, W.H.; Levanduski, M.J.

Source: THE PROGRESSIVE FISH CULTURIST 52(1):51-53 (1990).

Language: English

DNAL Call No.: 157.5 P94

Descriptors: freezing storage; sperm; genetics; biotechnology; natural populations; cultured organisms; fish culture; anadromous species; biological fertilization; hatching

**Abstract:**

This investigation determines if cryopreservation of salmonid sperm can be used successfully under normal field conditions. Sperm were collected from natural or wild steelhead (anadromous rainbow trout (*Oncorhynchus mykiss*)) trapped on two Lochsa River, Idaho, tributaries during spring 1987. Semen was frozen in liquid nitrogen and stored until spring 1988, when it was thawed, and the sperm were used to fertilize eggs of stock females from Dworshak National Fish Hatchery, Ahsahka, Idaho. Of the 44,676 eggs used from six females, 10,404 developed to the eyed stage and 10,254 embryos hatched.

96

*Fish Embryo Cell Cultures for Derivation of Stem Cells and Transgenic Chimeras. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Collodi, P.; Kamei, Y.; Barnes, D.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 63.

Language: English

Descriptors: genetics; cell culture; fish culture

**Abstract:**

In work with fish embryo cell cultures we found a potent mitogenic activity (M.W. 25,000; pI 5.2) in extracts of trout embryos. We used this activity for multipassage culture of trout and zebrafish blastula-derived embryo cells and also derived zebrafish cultures from haploid embryos. We introduce stably-integrated exogenous marker genes into fish embryo cell lines by plasmid transfection and selection in vitro, and are evaluating the potential of genetically marked zebrafish embryo cell lines as pluripotent embryonal stem cells.

97

*Recent Advances in the Development of Growth Acceleration Biotechnologies for Salmonids. 2.*

*International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Donaldson, E.M.; McLean, E.; Souza, L.M.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 58.

Language: English

Descriptors: growth; aquaculture techniques; fish culture

**Abstract:**

The length of the production cycle and the size of the marketed product are major factors in the economic viability of finfish aquaculture systems. Biologically active proteins and peptides, anabolic steroids and thyroid hormones have all been tested for their ability to accelerate growth in fish. In particular, success has been achieved in the acceleration of growth in salmonid fishes by administration of recombinant somatotropins. We have now succeeded in accelerating growth in salmonids both by oral administration of somatotropin and by the

intraperitoneal implantation of a controlled release device. Future research directions include the search for more potent analogue from the somatotropin family and further laboratory and farm scale trials of controlled administration strategies.

98

*Growth Enhancement in Transgenic Atlantic Salmon by the Use of an "All Fish" Chimeric Growth Hormone Gene Construct*

Du, S.J.; Gong, Z.; Fletcher, G.L.; Shears, M.A.; King, M.J.; Idler, D.R.; Hew, C.L.

Source: BIOTECHNOLOGY 10(2):176-181 (1992).

Language: English

DNAL Call No.: Q320.B56

Descriptors: growth; genes; hormones

**Abstract:**

We have developed an "all fish" growth hormone (GH) chimeric gene construct by using an antifreeze protein gene (AFP) promoter from ocean pout linked to a chinook salmon GH cDNA clone. After microinjection into fertilized, nonactivated Atlantic salmon eggs via the micropyle, transgenic Atlantic salmon (*Salmo salar*) were generated. The presence of the transgene was detected by polymerase chain reaction (PCR) using specific oligonucleotide primers. A number of these transgenic fish showed dramatic increases in their growth rate. At one year old, the average increase of the transgenic fish was 2 to 6 fold and the largest transgenic fish was 13 times that of the average non-transgenic control.

99

*Gene Transfer of Growth Hormone in the Gilthead Sea Bream (*Sparus aurata*) and Characterization of its Pregrowth Hormone cDNA. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Funkenstein, B.; Cavari, B.; Moav, B.; Harari, O.; Chen, T.T.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 93.

Language: English

Descriptors: biotechnology; fish culture; genetics; fish eggs; *Salmo gairdneri*

**Abstract:**

In order to develop a fast growing strain of fish, we have microinjected a recombinant plasmid containing RSV-LTR linked to rainbow trout GH2 cDNA into fertilized eggs of the gilthead sea bream (*Sparus aurata*). DNA samples prepared from fins and/or blood of "transgenic" fish were analyzed by dot blot and Southern blot hybridization using the RSV-LTR as a probe. A GH cDNA was also isolated from a cDNA library of the gilthead sea bream pituitary RNA. The nucleotide sequence of *Sparus* GH cDNA is 928 nt long (including a poly (A) tail of 55 nt) and it encodes a pre-GH polypeptide of 204 amino acid residues.

100

*Genetics and Reproduction in Fish Culture*

Gall, G.A.E.

Source: JOURNAL OF ANIMAL SCIENCE 69(10):4216-4220 (1991).

Language: English

DNAL CALL NO.: 49 J82

Descriptors: fish culture; hormones; genetic improvement; reproduction; biotechnology

**Abstract:**

Fish genetics has made major strides during the past 20 yr due both to improvements in the ability of fish culturists to manage reproduction and to deliberate experimentation and

application. The general finding has been that the quantitative genetics of fish differ little from those of other animals and that the applications of animal improvement techniques are similar for fish and other animals. In addition, a number of novel techniques, such as ploidy manipulation and sex reversal, are relatively easy to achieve with a number of fish species. As a result, some very specialized approaches to research have been possible, and applications to fish production seem to be limited only to the imagination of the breeder. However, only limited application has occurred over a major portion of the industry, and genetic improvement of stocks has been achieved in very few instances. The reason for this apparent dichotomy between opportunity and reality seems to be related to the industry's lack of emphasis on genetic improvement.

101

*Cryopreservation of Rainbow Trout Spermatozoa. 8. Annual Meeting of the Aquaculture Association of Canada; St. Andrews, New Brunswick, Canada; June 1991*

Gallant, R.K.; McNiven, M.A.

Source: BULLETIN (AQUACULTURE ASSOCIATION OF CANADA) 91(3):25-27 (1991).

Language: English

DNAL Call No.: SH37.B8

Descriptors: fish culture; biotechnology; freezing storage; sperm

Abstract:

Dimethylacetamide (DMA) was evaluated as a cryoprotectant for rainbow trout (*Oncorhynchus mykiss*) spermatozoa on the basis of motility, percent dead sperm, enzyme leakage and fertility of frozen-thawed spermatozoa. DMA performed significantly better ( $p < 0.05$ ) than the conventional dimethylsulfoxide (DMSO) by all evaluation methods. An extender comprised of 0.137 M NaCl, 0.011 M KCl, 0.004 M  $\text{Na}_2\text{HPO}_4$  multiplied by  $7\text{H}_2\text{O}$  and 7.5 g/L 1-alpha-lecithin and 10% DMA showed promising results as a diluent (extender and cryoprotectant) for rainbow trout spermatozoa. Future evaluations of new diluents should be carried out by several in vitro techniques and fertility to present a complete assessment of the diluent's ability to cryopreserve sperm cells.

102

*Survival and Integration Rate of Channel Catfish and Common Carp Embryos Microinjected with DNA at Various Developmental Stages*

Hayat, M.; Joyce, C.P.; Townes, T.M.; Chen, T.T.; Powers, D.A.; Dunham, R.A.

Source: AQUACULTURE 99(3-4):249-256 (1991).

Language: English

DNAL Call No.: SH1.A6

Descriptors: ictalurus-punctatus; cyprinus-carpio; fish; aquaculture; transgenic

Abstract:

Channel catfish, *Ictalurus punctatus*, and common carp, *Cyprinus carpio*, were microinjected with DNA at early, intermediate or late one-cell stage, two-cell stage or four-cell stage to determine the most appropriate developmental stage for maximizing the production of transgenic individuals, a product of survival multiplied by integration rate. Survival (hatching rate) decreased ( $P < 0.05$ ,  $r = -0.81$ ) when channel catfish embryos were microinjected at later stages compared to earlier stages. Survival relative to non-microinjected controls was 65.0, 43.6, 38.5, 27.4, and 35.9% and integration rate was 2.1, 1.5, 15.2, 12.5 and 7.7% for early one-cell, intermediate one-cell, late one-cell, two-cell and four-cell stages, respectively. Cell stage at time of microinjection did not affect ( $P > 0.05$ ,  $r = -0.52$ ) survival in common carp and survival of microinjected common carp embryos was higher ( $P < 0.01$ ) than survival for channel catfish embryos. Survival relative to non-microinjected controls was 88.9, 85.3, 91.5, 85.3, and 83.4% and integration rate was 8.3, 1.0, 0.0, 11.5 and 1.7% for early one-cell,



intermediate one-cell, late one-cell, two-cell and four-cell stages, respectively, of common carp. The late one-cell and the two-cell stage were the most appropriate stages for maximizing production of transgenic channel catfish and common carp, respectively.

**103**

*Methylation Status of the MTrGH Gene Construct in Transgenic Rainbow Trout (Oncorhynchus mykiss).* 2. *International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Iyengar, A.; MacLean, N.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 91.

**Language:** English

**Descriptors:** fish culture; biotechnology; genetics; *Salmo gairdneri*

**Abstract:**

DNA methylation has often been implicated in the regulation of gene expression. Methylation studies were carried out on adult rainbow trout identified transgenic for the MTrGH gene construct (mouse metallothionein-1 promoter spliced to the rat growth hormone gene). DNA from different tissues of the fish was digested with the methylation sensitive restriction enzyme isoschizomers, MspI and HpaII. No major tissue-specific differences were detected although several sites were found to remain consistently methylated.

**104**

*The Use of Homologous Genes for the Obtaining of Transgenic Fish.* 2. *International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Kavsan, V.M.; Koval, A.P.; Grebenjuk, V.V.; Palamarchuk, A.J.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 78.

**Language:** English

**Descriptors:** hormones; genetics; growth; insulin; Pisces; fish culture

**Abstract:**

There are some evidences of correlation of insulin level in blood of fish and their growth. Taking this correlation into account the authors inoculated chum salmon preproinsulin in the fertilized eggs of loach, carp, zebrafish and trout and obtained the transgenic animals. The influence of exogene on growth and the possible increase of carbohydrate utilization will be discussed. For the obtaining of transgenic fish with the help of homologous genes from the chum salmon genomic bank the authors isolated and sequenced the gene of insulin-like growth factor I as well as the growth hormone genes. The comparison of these genes with the known orthological genes are reported.

**105**

*Gynogenesis in Common Carp (Cyprinus carpio L.).* 2. *The Production of Homozygous Gynogenetic Clones and F sub(1) Hybrids*

Komen, J.; Bongers, A.B.J.; Richter, C.J.J.; van Muiswinkel, W.B.; Huisman, E.A.

**Source:** AQUACULTURE 92(2-3):127-142 (1991).

**Language:** English

**DNAL Call No.:** SH1.A6

**Descriptors:** fish culture; clones; hybrids; inbreeding; biotechnology

**Abstract:**

Homozygous gynogenetic fry of common carp (*Cyprinus carpio*) were produced by heat-shocking eggs, activated with UV-irradiated sperm during metaphase of the first mitosis. Consistent yields of 5-15% viable, gynogenetic fry were obtained when eggs were shocked at



40 degree C for 2 min, 28-30 min after fertilization. Homozygous inbred strains were produced by heterozygous gynogenetic reproduction (second polar body retention) of homozygous gynogenetic females, while F<sub>1</sub> hybrids were produced by crossing these females with homozygous gynogenetic male siblings.

106

*In Vitro and In Vivo Gene Transfer in Fish. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Leung, F.; Welt, M.; Chandler, D.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 78.

**Language:** English

**Descriptors:** genes; genetics; fish culture; methodology; fish eggs

**Abstract:**

We are studying exogenous gene transfer and gene expression in fish both in vivo and in vitro. For the in vitro studies, a transient transfection assay was used to examine the effects of mammalian and fish promoters, fish cell lines, and form of a reporter gene on gene expression. We use the genomic (bGH) and intronless bovine growth hormone (bGH- Delta 1) as reporter genes; mouse fibroblast, RTG-2, RTH-149, BB, EPC as recipient cells, mouse (mMT) and trout metallothionein (rtMT) gene promoters, and a bGH radioimmunoassay for detection in the in vitro transfection assays. For the in vivo studies, we have developed a simple, efficient microinjection technique for gene transfer into fertilized rainbow trout (*Oncorhynchus mykiss*) eggs.

107

*Advances in Fin Fish Induced Breeding--from Laboratory to Field. 1. International Marine Biotechnology Conference (IMBC '89); Tokyo (Japan); 4-6 Sept 1989*

Little, J.M.; Dawson, J.A.

**Source:** PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '89). 1989, p. 43.

**Language:** English

**Descriptors:** fish culture; induced breeding; hormones

**Abstract:**

Not many years ago induced breeding of fin fish was a variable, species specific technique to produce eggs and sperm. Today it is highly consistent and predictive. The pressures to produce fish seed for culture mount as traditional wild stocks deplete and consumer market demand increases. As culture becomes more sophisticated, seed production for quantity, timeliness and genetic selection is intensified. Today, a fourth generation hormonal induction system has succeeded in spawning many fish species. These include the three Indian major carps, grass carp, common carp, mud carp, silver carp, black carp, bighead carp, loach, Puntius, Pacu, catfish, salmon, trout, sturgeon and striped bass. A discussion on the state-of-the-art in induced breeding at the research level leads into the adaptation of this research to the real world of aquaculture.

108

*Techniques to Produce 100% Male Tilapia*

Pandian, T.J.; Varadaraj, K.

**Source:** NAGA 13(3):3-5 (1990).

**Language:** English

**DNAL Call No.:** SH332.I6

**Descriptors:** fish culture; selective breeding; monosex culture; aquaculture techniques; sex reversal

**Abstract:**

A discussion is presented on endocrine sex reversal and chromosome manipulation techniques for producing 1005 male tilapia. Details are given of methods for the administration of steroids and the induction of ploidy.

**109**

*Factors Affecting Survival and Integration Following Microinjection of Novel DNA into Rainbow Trout Eggs. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Penman, D.J.; Beeching, A.J.; Penn, S.; MacLean, N.

Source: AQUACULTURE 85(1-4):35-50 (1990).

Language: English

DNAL Call No.: SH1.A6

Descriptors: biotechnology; fish culture; genetics; fish eggs; biological development; survival

**Abstract:**

The authors have attempted to assess the relative merits of different injection techniques, DNA concentrations, buffers and DNA structure. The design of such experiments has also been drawn from the data available about the production of transgenic mice. The authors present results from DNA microinjection experiments in the rainbow trout (*Salmo gairdneri*). Using one basic protocol, each of the parameters was varied and its effects assessed on survival during development and on integration frequency in the resultant fry.

**110**

*Patterns of Transgene Inheritance in Rainbow Trout (Oncorhynchus mykiss)*

Penman, D.J.; Iyengar, A.; Beeching, A.J.; Rahman, A.; Sulaiman, Z.; MacLean, N.

Source: MOL. REPROD. DEV. 30(3):201-206 (1991).

Language: English

DNAL Call No.: QP251.M64

Descriptors: DNA; hybridization; fish culture; genetics; *Salmo gairdneri*

**Abstract:**

There have been very few studies of the inheritance of introduced genes (transgenes) in fish. The authors have followed the inheritance of the mammalian fusion gene MTrGH from founder generation transgenics (originating from eggs microinjected with the MTrGH DNA) to offspring in crosses with control fish (rainbow trout, *Oncorhynchus mykiss*). Initial screening of the founder generation transgenics was by analysing DNA from blood samples. Only three out of six fish which carried the novel gene in blood DNA transmitted it to their offspring, despite the presence of the gene in DNA extracted from the sperm of all four male fish in this group. The frequency of transgenics in the progeny groups from the three fish which transmitted the gene varied widely: in one of these groups more than one type of MTrGH restriction pattern was found. These results suggest widespread mosaicism in founder generation transgenics.

**111**

*Successful Gene Transfer and Expression of Novel Gene Constructs in Tilapia (Oreochromis niloticus). 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Rahman, M.A.; Sulaiman, Z.; MacLean, N.

Source: PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 94.

Language: English

Descriptors: fish eggs; fish culture; genetics; biotechnology

**Abstract:**

We here report the successful transfer of two different DNA constructs into one-cell stage

fertilized eggs of tilapia (*Oreochromis niloticus*) by microneedle injection. Variable numbers of copies of these transgenes are incorporated into the genomes of fish developing from injected eggs. Southern blotting patterns indicate genomic integration in approximately 20% of the fish developing from injected eggs. Introduction of DNA constructs in which a fish gene promoter is spliced to a bacterial CAT gene were used to determine levels of transgene expression.

112

*Recombinant DNA Techniques Applied to Fish Farming. 1. International Marine Biotechnology Conference (IMBC '89); Tokyo (Japan); 4-6 Sept 1989*

Renard, A.; Denis, C.; Dheur, I.; Dommes, J.; Lebecque, S.; Lecomte, C.; Poncin, A.; Smal, J.; Thiry, M.; et al.

**Source:** PROGRAM OF THE FIRST INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '89). 1989, p. 75.

**Language:** English

**Descriptors:** fish culture; biotechnology; DNA; viral diseases; septicemia; clones; vaccines; antibodies; hormones; growth regulators

**Abstract:**

The cDNA coding for the surface protein of the viral hemorrhagic septicemia virus has been cloned in *Escherichia coli*. The entire and truncated forms of this protein have been expressed in *E. coli* and *Saccharomyces cerevisiae* and have been used to vaccinate trout by injection, dipping or analintubation. The protection has been checked by apparition of specific antibodies and viral challenges. The cDNA coding for the growth hormones and prolactins of trout and tilapia have been cloned and expressed in *E. coli* and *S. cerevisiae*. The recombinant proteins have been purified to near homogeneity. Their biological activities have been checked into trouts (injection and addition into food).

113

*Experimental Administration of Recombinant Bovine Growth Hormone to Juvenile Rainbow Trout (*Salmo gairdneri*) by Injection or by Immersion*

Schulte, P.M.; Down, N.E.; Donaldson, E.M.; Souza, L.M.

**Source:** AQUACULTURE 76(1-2):145-156 (1989).

**Language:** English

**DNAL Call No.:** SH1.A6

**Descriptors:** growth regulators; hormones; biotechnology; fish culture; juveniles; condition factor; aquaculture techniques

**Abstract:**

The ability of recombinant bovine growth hormone 21K (rbGH-21K) to accelerate the growth of juvenile rainbow trout (*Salmo gairdneri*) was investigated, and the potential of bath immersion as a method of administration of growth hormone was assessed. Injection of 10 µg/g rbGH-21K every 2 weeks for 8 weeks resulted in a highly significant increase in the rate of growth in both weight and length as well as an increase in condition factor. Administration of rbGH-21K by immersion at a dosage of 100 mg/l for 4 h every 5 days resulted in a significant increase in specific growth rate relative to similarly treated controls during 8-week treatment period, although the effect was minimal when compared to the injected group. Comparison of growth in immersed versus non-immersed controls indicated that the immersion protocol itself inhibited growth. Modifications to the immersion protocol which may improve its effectiveness are discussed.



114

*Genetic Variation in Quantitative Characters of Meiotic- and Mitotic-gynogenetic Diploid ayu, Plecoglossus altivelis. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Taniguchi, N.; Hatanaka, H.; Seki, S.

Source: AQUACULTURE 85(1-4):223-233 (1990).

Language: English

DNAL Call No.: SH1.A6

Descriptors: biotechnology; fish culture; genetics; phenotypic variations; animal morphology; isoenzymes; selective breeding

Abstract:

Two types of gynogenetic diploids were artificially induced in the ayu, *Plecoglossus altivelis*, meiotic-G2N and mitotic-G2N. The success of inducing these two types of gyno-genetic diploids was verified by an isozyme marker at the Gpi-1 locus. This showed a high percentage of heterozygosity in the meiotic-G2N and 0% heterozygosity in the mitotic-G2N. There was a general tendency for the variance and coefficient of variation of the quantitative characters to be always large in the mitotic-G2N, small in the control-N and intermediate in the meiotic-G2N. These results are partly explained by formulae showing the correlation of increased phenotypic variance with increased genetic variance, increased coefficient of inbreeding, and deleterious effects of inbreeding.

115

*Genetic Consequences of Salmonid Egg Fertilization Techniques. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Withler, R.E.

Source: AQUACULTURE 85(1-4):326 (1990).

Language: English

DNAL Call No.: SH1.A6

Descriptors: biotechnology; fish culture; genetics; aquaculture techniques; hatcheries; biological fertilization; population number

Abstract:

Hatchery fertilization procedures for salmonid fishes were examined experimentally in an attempt to define a methodology that would maximize the effective population size for a given broodstock size by avoiding or reducing sperm competition among males. The contributions to fertilization (potency) of individual male chinook salmon (*Oncorhynchus tshawytscha*) whose milt was added sequentially to eggs, or was pooled before application, were determined by electrophoresis of the resulting progeny. The most potent male sired more than 40% of the offspring even in crosses in which his milt was applied last. These results indicate that when a male:female ratio of 1:1 or lower is maintained in a hatchery broodstock, no pooling or sequential addition of milt can be carried out without reducing effective population size. If male fertility problems in a stock dictate the use of more than one male per cross, genetic variation will be maximized by maintaining a broodstock male:female ratio of greater than one.

116

*Retrospects and Prospects of Fish Genetics and Breeding Research in China. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Wu, C.

Source: AQUACULTURE 85(1-4):61-68 (1990).

Language: English

DNAL Call No.: SH1.A6



**Descriptors:** biotechnology; fish culture; genetics; selective breeding; acclimatization; hybridization; polyploids

**Abstract:**

In this paper, fish genetics and breeding research in China over the last decade are reviewed. It includes acclimatization, hybridization, gynogenesis, artificially induced polyploidy, somatic cell breeding and gene transfer. Two wild species, *Megalobrama amblycephala* and *Plagiognathops microlepis*, were acclimatized. Five carp hybrids of obvious vigour were widely applied to fish culture. After looking through the results of hybridization of distantly related combinations of Chinese freshwater fish, three factors causing incompatibility were determined. By means of artificial gynogenesis and sex reversal, three pure lines of carp were established. Using serial nuclear transplantation techniques, Chinese genetics obtained a "tube fish". A number of novel growth hormone gene transgenic fishes, including goldfish, common carp, mirror carp, loach, crucian carp, mud carp and blunt snout bream, have been produced. The novel gene has been clearly detected in adult and second generation samples.

117

*Transfer of the Gene for Neomycin Resistance into Goldfish, Carassius auratus. 3. International Symposium on Genetics in Aquaculture; Trondheim (Norway); 20-24 June 1988*

Yoon, S.J.; Hallerman, E.M.; Gross, M.L.; Liu, Z.; Schneider, J.F.; Faras, A.J.; Hackett, P.B.; Kapuscinski, A.R.; Guise, K.S.

**Source:** AQUACULTURE 85(1-4):21-33 (1990).

**Language:** English

**DNAL Call No.:** SH1.A6

**Descriptors:** biotechnology; fish culture; genetics

**Abstract:**

A recombinant DNA construct was microinjected into newly fertilized, dechorionated goldfish (*Carassius auratus*) eggs. The neo gene confers resistance to the neomycin analog drug G-418. Results of Southern blot analyses were consistent with incorporation of single or multiple copies of the gene into the genomic DNA of one examined fish. RNA dot-blot analysis indicated transcription within a piscine genome. The utility of the neo gene as a selectable marker for transgenic fish was evaluated by G-418 selection on newly hatched and juvenile fish but proved inconclusive. Reasons for the discrepancy between neo expression and G-418 selection results are discussed.

118

*Biotechnologies for the Manipulation of Reproduction in Fish Farming. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Zohar, Y.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 57.

**Language:** English

**Descriptors:** sexual reproduction; spawning; genes; fish culture; reproductive cycle; sex hormones; induced breeding; aquaculture techniques; intensive culture

**Abstract:**

Understanding the molecular and physiological basis of fish reproduction and spawning, and developing technologies for the manipulation of these processes are basic requirements for the intensification of commercial fish farming. Studying the neuroendocrine mechanisms involved in the regulation of gametogenesis led to the elucidation of the interactions between the environment, brain, pituitary and gonadal hormones in the control of ovulation and spawning.

These data led to the introduction of efficient hormonal treatments to induce year round spawning in many farmed fish, based on the use of the brain peptide gonadotropin releasing hormone (GnRH).

## Field Release Studies

119

*Recent Developments in Public Policies Regulating the Development of Transgenic Fishes. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*  
Hallerman, E.M.; Kapuscinski, A.R.

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 78.

**Language:** English

**Descriptors:** government policy; health and safety; genetics

**Abstract:**

Development of public policies regulation use of genetically modified organisms is currently at a pivotal stage. In the United States, proposed environmental release policies publicized by the Office of Agricultural Biotechnology of the Dept. of Agriculture appear to conflict with policy guidelines recently discussed by the Biotechnology Working Group of the Council on Economic Competitiveness of the White House Office of Science and Technology Policy. Resolution of this conflict will have great bearing on the rate of progress and ecological safety of field tests with transgenic fish in the United States. State governments are also undertaking development of biotechnology regulations, primarily in response to persistent gaps in environmental protection caused by unresolved policy conflicts at the federal levels.

120

*Transgenic Fish and Public Policy: Patenting of Transgenic Fish*

Hallerman, E.M.; Kapuscinski, A.R.

**Source:** FISHERIES 15(1):21-24 (1990).

**Languages:** English

**DNAL Call No.:** SH1.F54

**Descriptors:** fish culture; clones; genetics; breeding

**Abstract:**

Following the granting of the first patent for a transgenic animal, public debate has arisen over a number of contentious patenting issues, including the very patentability of higher life forms. Against the background of the legal history regarding patentability of novel animals and the viewpoints of the various interest groups, we identify key policy questions currently at issue in the U.S. Congress and the U.S. Patent and Trademark Office. Finally, we propose positions that the American Fisheries Society might advocate at this critical moment in the determination of national animal patenting policies which are likely to impact the community of fisheries professionals.

121

*Transgenic Fish and Public Policy: Regulatory Concerns*

Hallerman, E.M.; Kapuscinski, A.R.

**Source:** FISHERIES 15(1):12-20 (1990).

**Language:** English

**DNAL Call No.:** SH1.F54

**Descriptors:** government policy; genetics; fish culture; biotechnology; fish

**Abstract:**

The present status of public policies regarding laboratory production, field testing, and distribution and final use of transgenic animals is reviewed from the perspective of fisheries professionals. Unsettled policy issues center upon distribution and final use of transgenic fishes. Policy positions regarding regulation of the various stages of production of transgenic fishes are recommended for adoption by fisheries professionals. As each stage poses a different level of risk to the environment and is subject to a different set of regulations, each stage is treated separately in the discussion below.

**122***Implications of Introduction of Transgenic Fish into Natural Ecosystems*

Kapuscinski, A.R.; Hallerman, E.M.

**Source:** CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES 48(suppl. 1):99-107 (1991).

**Language:** English

**DNAL Call No.:** 442.9 C16J

**Descriptors:** environmental impact assessment; aquatic wildlife management; fisheries management policy

**123***Transgenic Fish and Public Policy: Anticipating Environmental Impacts of Transgenic Fish*

Kapuscinski, A.R. and Hallerman, E.M.

**Source:** FISHERIES 15(1):2-11 (1990).

**Language:** English

**DNAL Call No.:** SH1.A6

**Descriptors:** genetics; environmental impact; biotechnology; introduced species; fish culture; government policy

**Abstract:**

Transfer of novel genes into fishes introduces a number of contentious issues into public policy debate among fisheries scientists and regulatory authorities. In the context of the technical status of development of transgenic strains of fishes, we discuss anticipated ecological impacts of releasing such fishes into natural environments. The major determinant of ecological impacts of transgenic fishes will be the phenotypic effect of the inserted genes. Three conceptual classes of phenotypic changes are anticipated, including changes in physiological rates, behavior, or tolerance of physical factors. We identify major research needs for formulation of quantitative risk analysis protocols and suggest items to include in a position statement on transgenic fishes for adoption by the American Fisheries Society.

**124***Genetic Manipulation of Freshwater Fish Developmental Status Significance and Ecological Risks*

Lukowicz, V.; Foerster, M.; Hilge, V.; Klupp, R.; Roesch, R.; Schultze, D.; Stein, H.; Ungemach, H.

**Source:** BAYERISCHES LANDWIRTSCHAFTLICHES JAHRBUCH 67(7):771-782 (1990).

**Language:** German

**DNAL Call No:** 18 L24

**Descriptors:** monosex fish; transgenic fish

**125***Development of Transgenic Fish as Models for Study of Aquatic Contaminants. 2. International Marine Biotechnology Conference (IMBC '91); Baltimore, MD (USA); 13-16 Oct 1991*

Winn, R.N. and Beneden, R.J. van

**Source:** PROGRAM AND ABSTRACTS. SECOND INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE (IMBC '91). 1991, p. 78.

**Language:** English

**Descriptors:** pollution monitoring; indicator species; pollution indicators; genetics

**Abstract:**

The development of transgenic organisms, genetically engineered to detect specific toxicants, is a novel approach to the study of aquatic toxicology. We are testing the hypothesis that reporter genes can be stably integrated and transmitted in fish, and expressed in response to specific chemical contaminants. We have made significant progress with prototype systems in which gene constructs have been microinjected into hundreds of fish embryos (ooplasmic injection, with *Oryzias latipes* and *Fundulus heteroclitus*). DNA extracted from fin clips of presumptive transgenic fish has been screened for the exogenous genes using PCR indicating -70% positive.



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